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Fig. 3

SDS-PAGE Analysis of
2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 Protein.

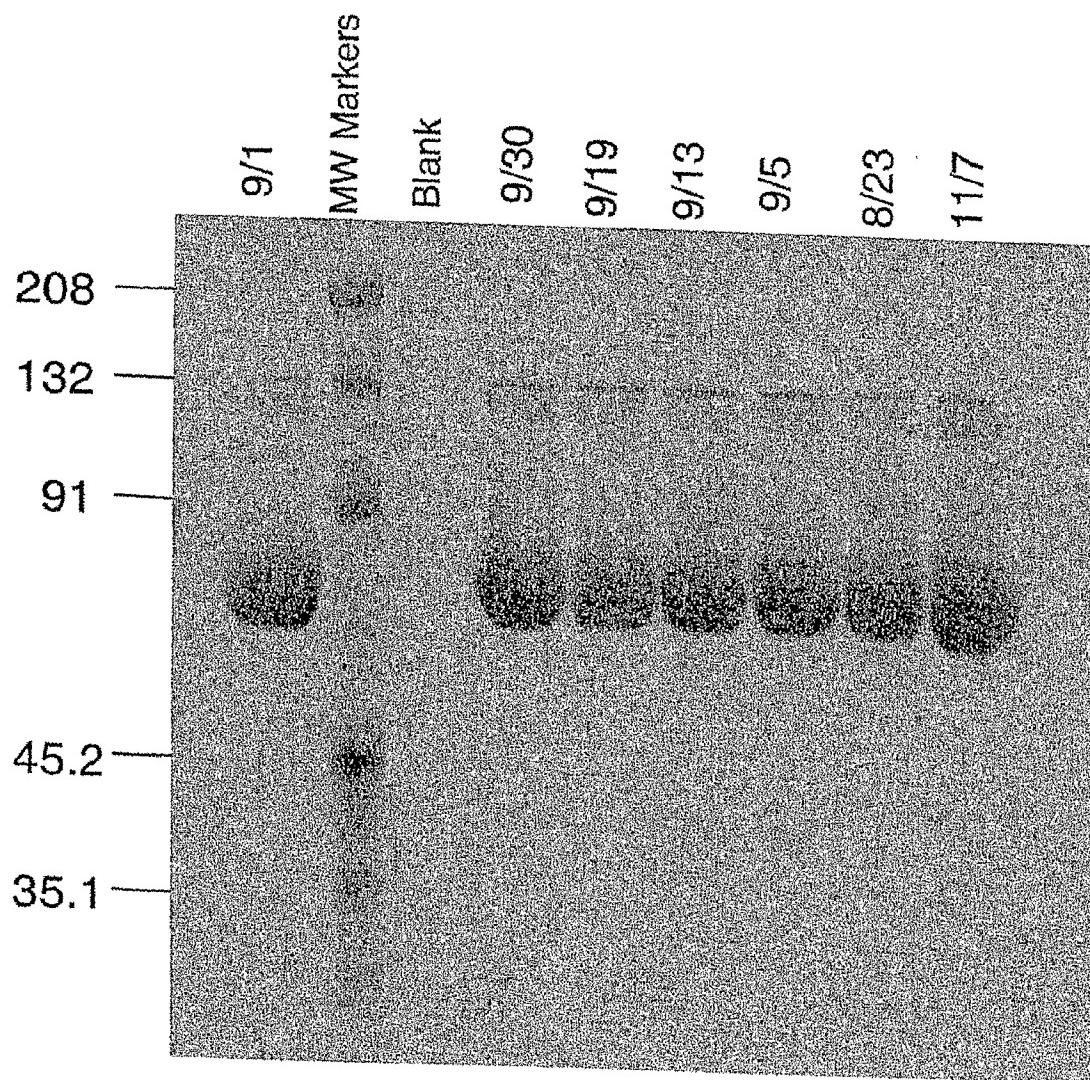


Fig. 4A

Complement Mediated B Cell Killing After Binding of
CD20-targeted 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3:

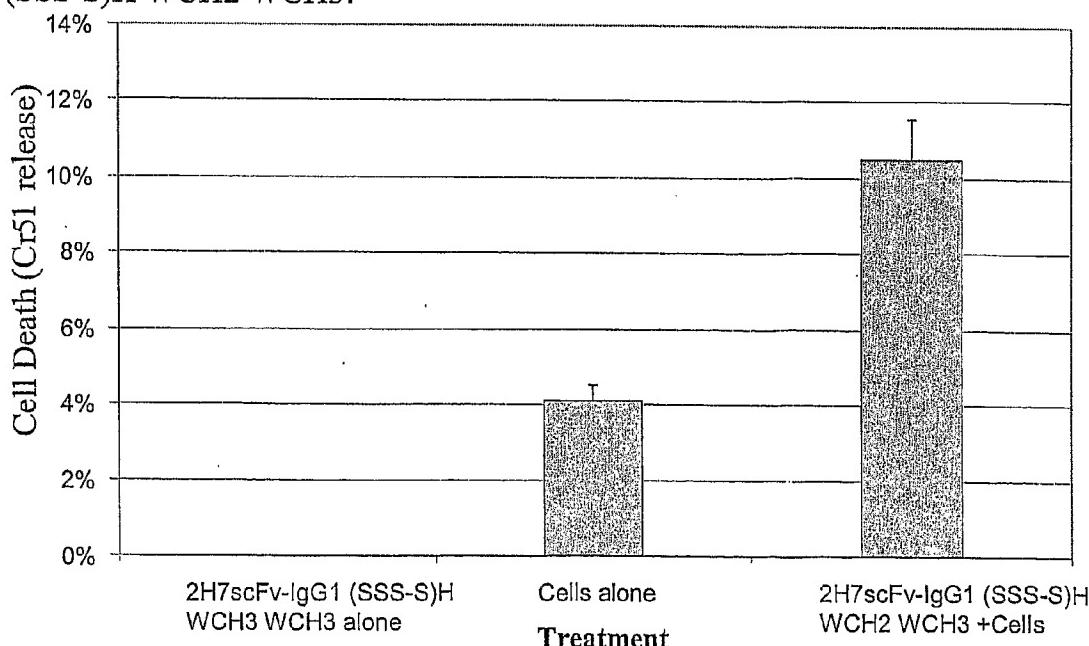
2H7scFv-Ig Concentration	RAMOS		BJAB	
	# live cells/total cells			
20 µg/ml + complement	-	0.16	-	0.07
5 µg/ml + complement	-	0.2	-	N.D.
1.25 µg/ml + complement	-	0.32	-	0.1
Complement alone	-	0.98	-	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Fig. 4B

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-IgG1 (SSS-S)H WCH2 WCH3:



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Fig. 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors
on B Cell Proliferation:

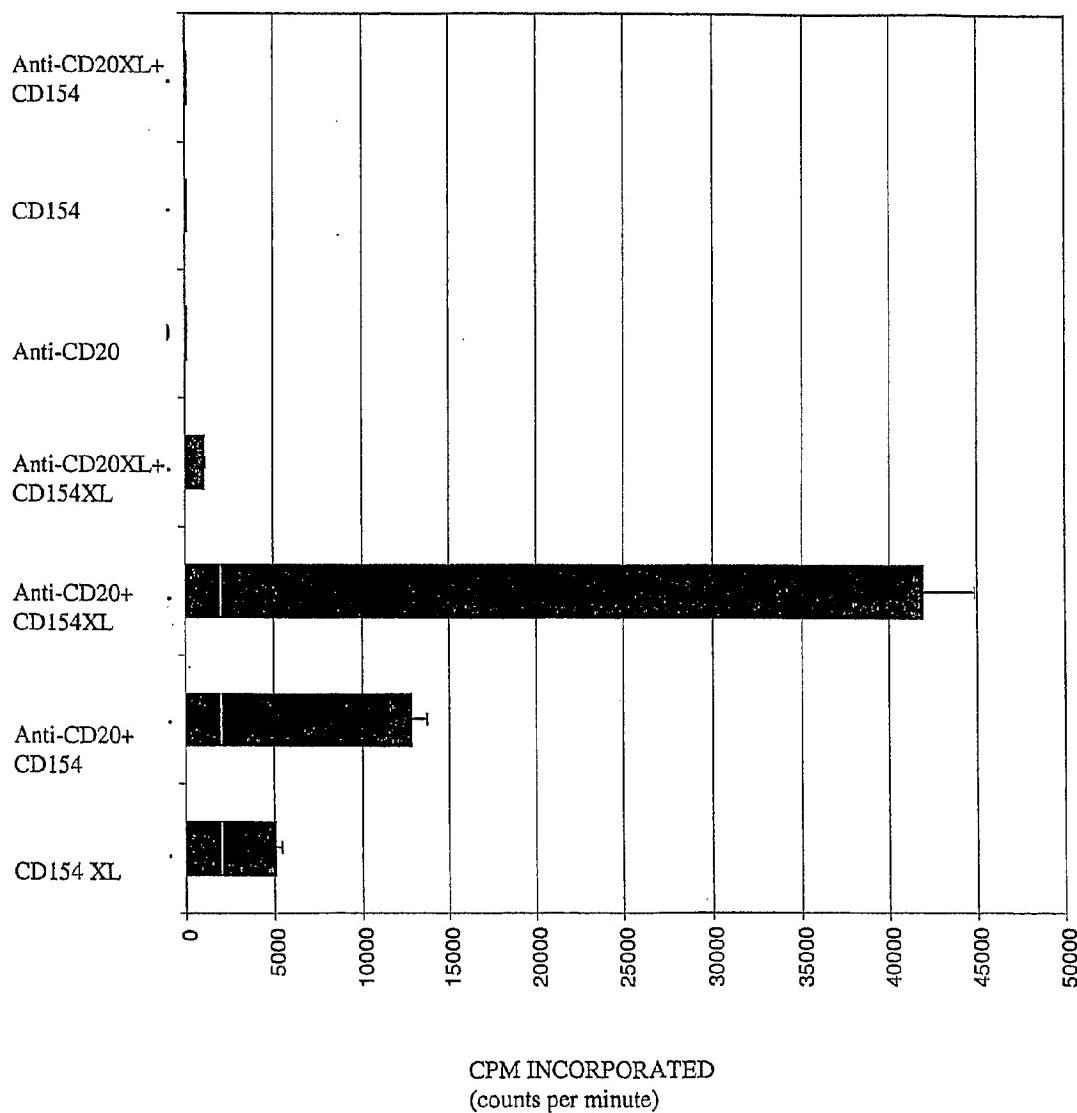


Fig. 6

Effect of Simultaneous ligation of CD20 and CD40
on CD95 and apoptosis.

Fig. 6A.

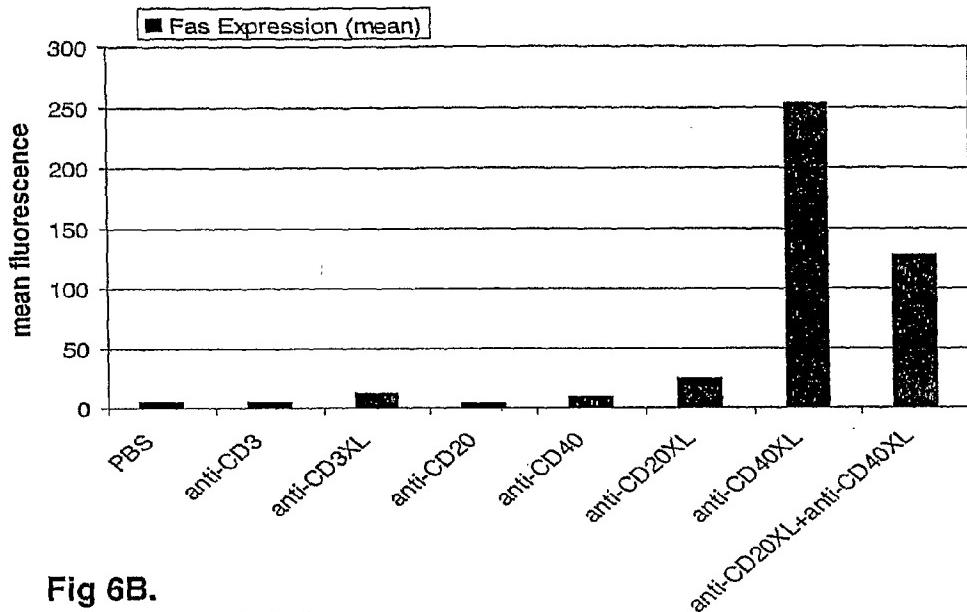
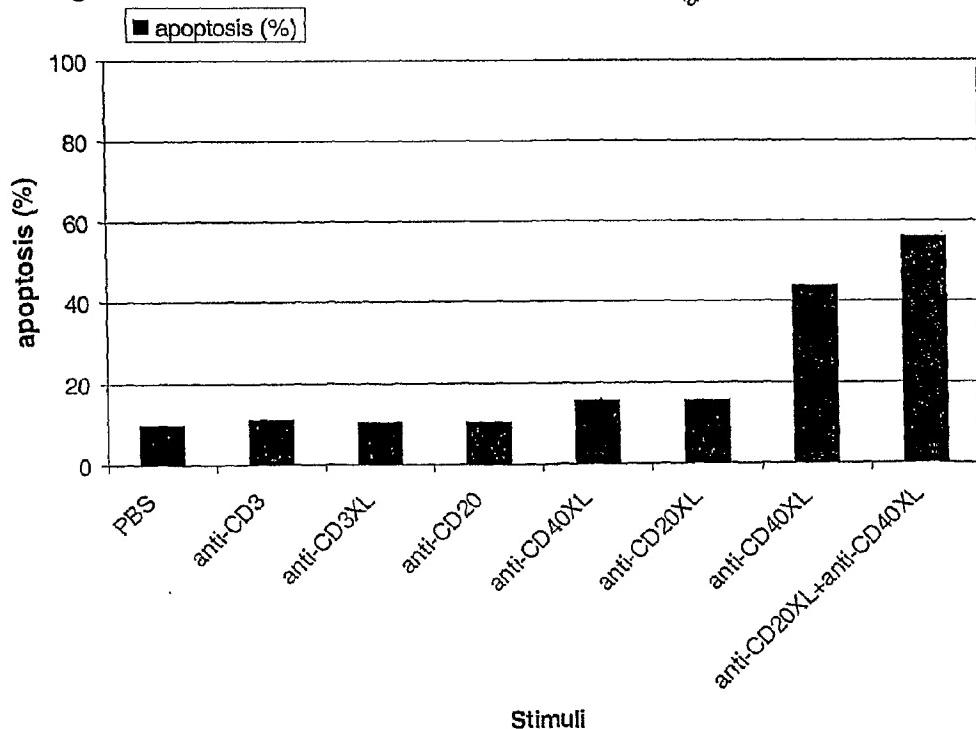


Fig 6B.



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Fig. 7A

2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide →

1 M D F Q V Q I F S F L L I S A S
 AAGCTTGCCG CC ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAAT CAGTGCTTCA

61 V I I A R G Q I V L S Q S P A I L S A S
 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

121 P G E K V T M T C R A S S S V S Y M H W
 CCAGGGGAGA AGGTCAACAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI

181 Y Q Q K P G S S P K P W I Y A P S N L A
 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTG ATGCCCATC CAACCTGGCT

241 S G V P A R F S G S G S G T S Y S L T I
 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

301 S R V E A E D A A T Y Y C Q Q W S F N P
 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA

(Gly₄Ser)₃ Linker →

361 P T F G A G T K L E L K G G G G S G G G
 CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT

421 2H7 V_H →
 G S G G G G S S Q A Y L Q Q S G A E L V
 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG

481 R P G A S V K M S C K A S G Y T F T S Y
 AGGCCTGGGG CCTCAGTGAA GATGTCTGCA AAGGCTCTG GCTACACATT TACCAGTTAC

541 N M H W V K Q T P R Q G L E W I G A I Y
 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

601 P G N G D T S Y N Q K F K G K A T L T V
 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

661 D K S S S T A Y M Q L S S L T S E D S A
 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

721 V Y F C A R V V Y Y S N S Y W Y F D V W
 GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

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Fig. 7A (continued)

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E
 GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E
 AGGAATCTTC ATGAAGATTG TGTATTCATG AAAACGATAAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
 AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAAG

BcII

961 D I M L N K E E T K K E N S F E M Q K G
 GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTGAAAT GCAAAAGGT

BcII

~~~~~

1021     D Q N P Q I A A H V I S E A S S K T T S  
 GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

1081     V L Q W A E K G Y Y T M S N N L V T L E  
 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCTGGAA

1141     N G K Q L T V K R Q G L Y Y I Y A Q V T  
 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

## HindIII

~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K
 TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAT GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
 CCTTGCAGGC AACAAATCCAT TCACCTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

~~~~~

1381     V F V N V T D P S Q V S H G T G F T S F  
 GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

## XbaI                  XbaI

~~~~~

~~~~~

1441     G L L K L E \* \* S R  
 GGCTTACTCA AACTCGAGTG ATAATCTAGA

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## Fig. 7B.

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```

HindIII      NcoI
~~~~~ ~~~~~~ 2H7 VL Leader Peptide →
M D F Q V Q I F S F L L I S A S
1 AAGCTTGC CG CC ATGGATTT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 VL →
V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCAACAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI
~~~~~
Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATTGCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA

(Gly4Ser)3 Linker →
P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 VH →
G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTCAA GATGTCCTGC AAGGCTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT

P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

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## Fig. 7B

human CD154/amino acid 108 →

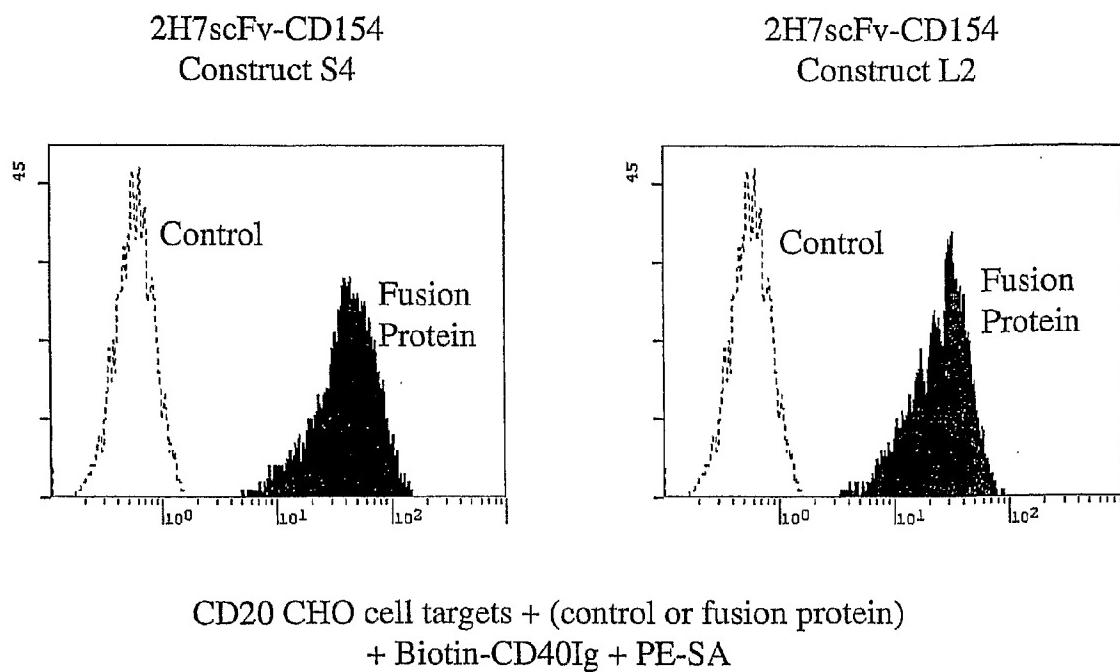
|      |                                         |                                                                     |
|------|-----------------------------------------|---------------------------------------------------------------------|
| 781  | BclI                                    | Bcl/Bam hybrid site                                                 |
|      | G T G T T V T V S D P E N S F E M Q K G | GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTGAAAT GCAAAAAGGT    |
|      | <hr/>                                   |                                                                     |
| 841  | BclI                                    |                                                                     |
|      | D Q N P Q I A A H V I S E A S S K T T S | GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT |
|      | <hr/>                                   |                                                                     |
| 901  | V L Q W A E K G Y Y T M S N N L V T L E | GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCCCTGGAA   |
|      | <hr/>                                   |                                                                     |
| 961  | N G K Q L T V K R Q G L Y Y I Y A Q V T | AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC   |
|      | <hr/>                                   |                                                                     |
| 1021 | HindIII                                 |                                                                     |
|      | S P G R F E R I L L R A A N T H S S A K | TTCTGTTCCA ATCGGGAAAGC TTGAGTC GCTCCATTAGCCAGCCT CTGCCTAAAG         |
|      | <hr/>                                   |                                                                     |
| 1081 | S P G R F E R I L L R A A N T H S S A K | TCCCCCGGTA GATTGAGAG AATCTTAATC AGAGCTGCAA ATACCCACAG TTCCGCCAAA    |
|      | <hr/>                                   |                                                                     |
| 1141 | P C G Q Q S I H L G G V F E L Q P G A S | CCTTGCAGGC ACAATCCAT TCACCTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG     |
|      | <hr/>                                   |                                                                     |
| 1201 | NcoI                                    |                                                                     |
|      | V F V N V T D P S Q V S H G T G F T S F | GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCCTT   |
|      | <hr/>                                   |                                                                     |
| 1261 | XbaI                                    | XbaI                                                                |
|      | G L L K L E * * S R                     | GGCTTACTCA AACTCGAGTG ATAATCTAGA                                    |

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Fig. 8

Simultaneous Binding of 2H7scFv-CD154  
Fusion Proteins to CD20 and CD40

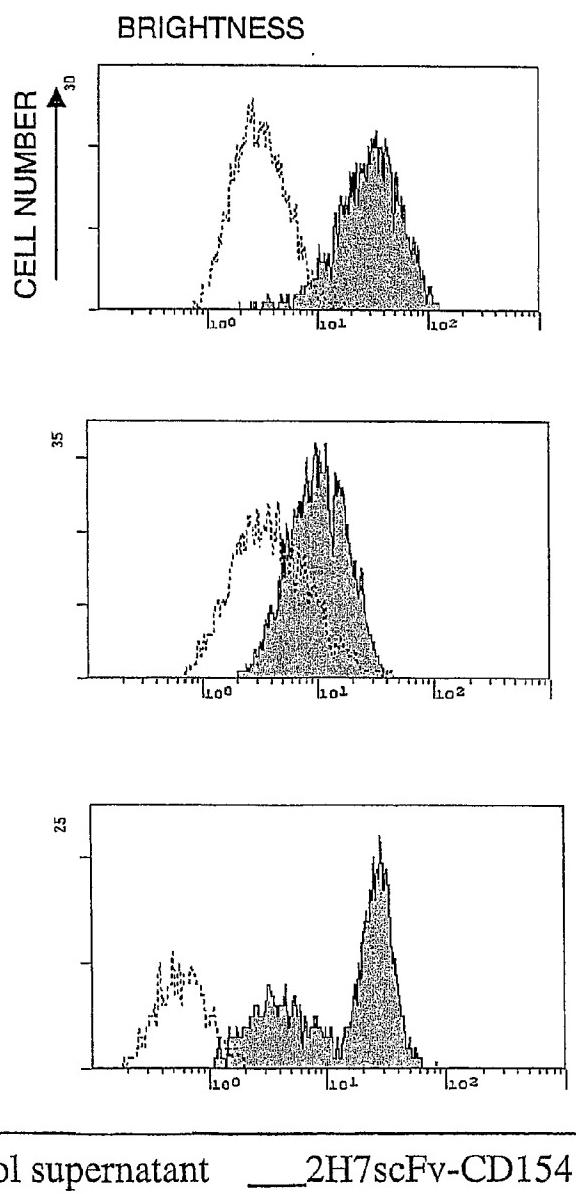


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Fig. 9

Induction of Apoptosis Measured by Binding of Annexin V after incubation with 2H7scFv-CD154



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Fig. 10

Proliferation of T51 B Cell Line After Incubation with 2H7 scFv-CD154 S4 or 2H7 scFv-CD154 L2 Constructs

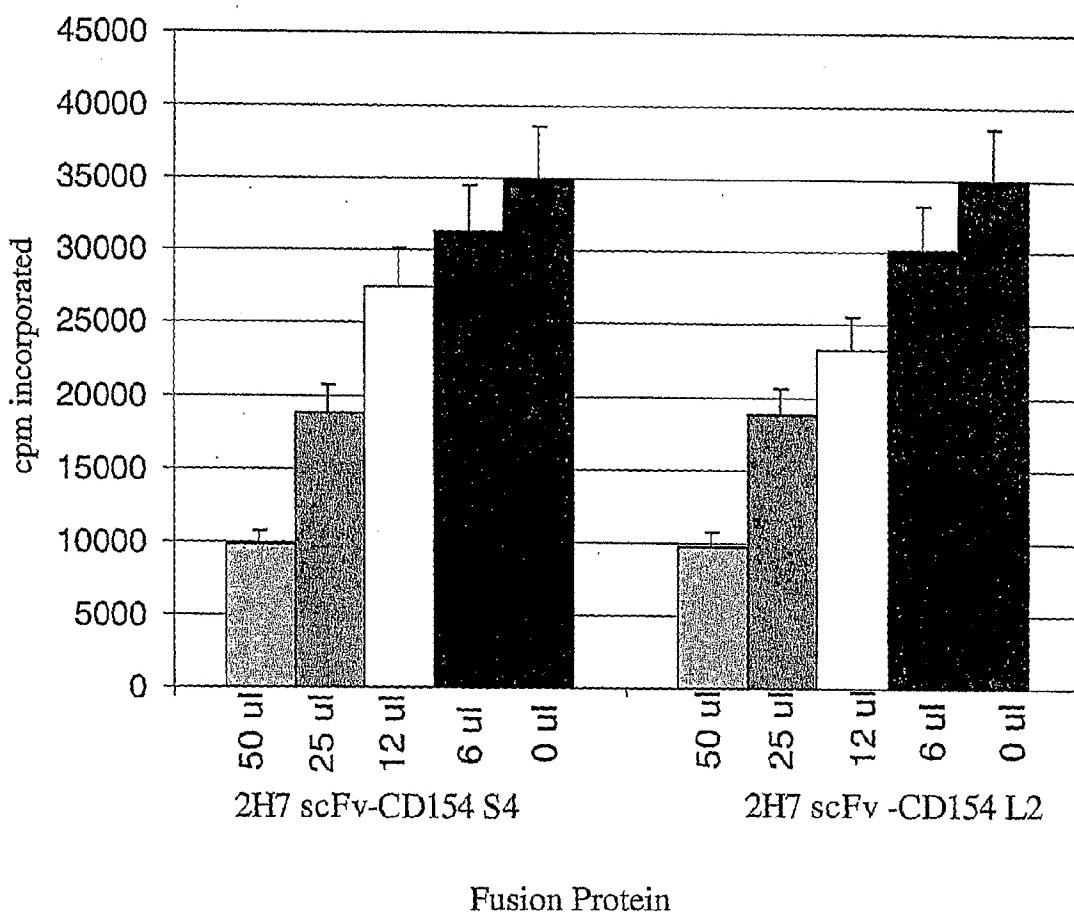


Fig. 11

### Schematic Representation of 2H7 scFvIg Constructs

2H7 scFvIgG (SSS-S)H WCH2 WCH3

OR 2H7 scFvIgG1 (SSS-S)H P238SCH2 WCH3 : 2H7 scFv

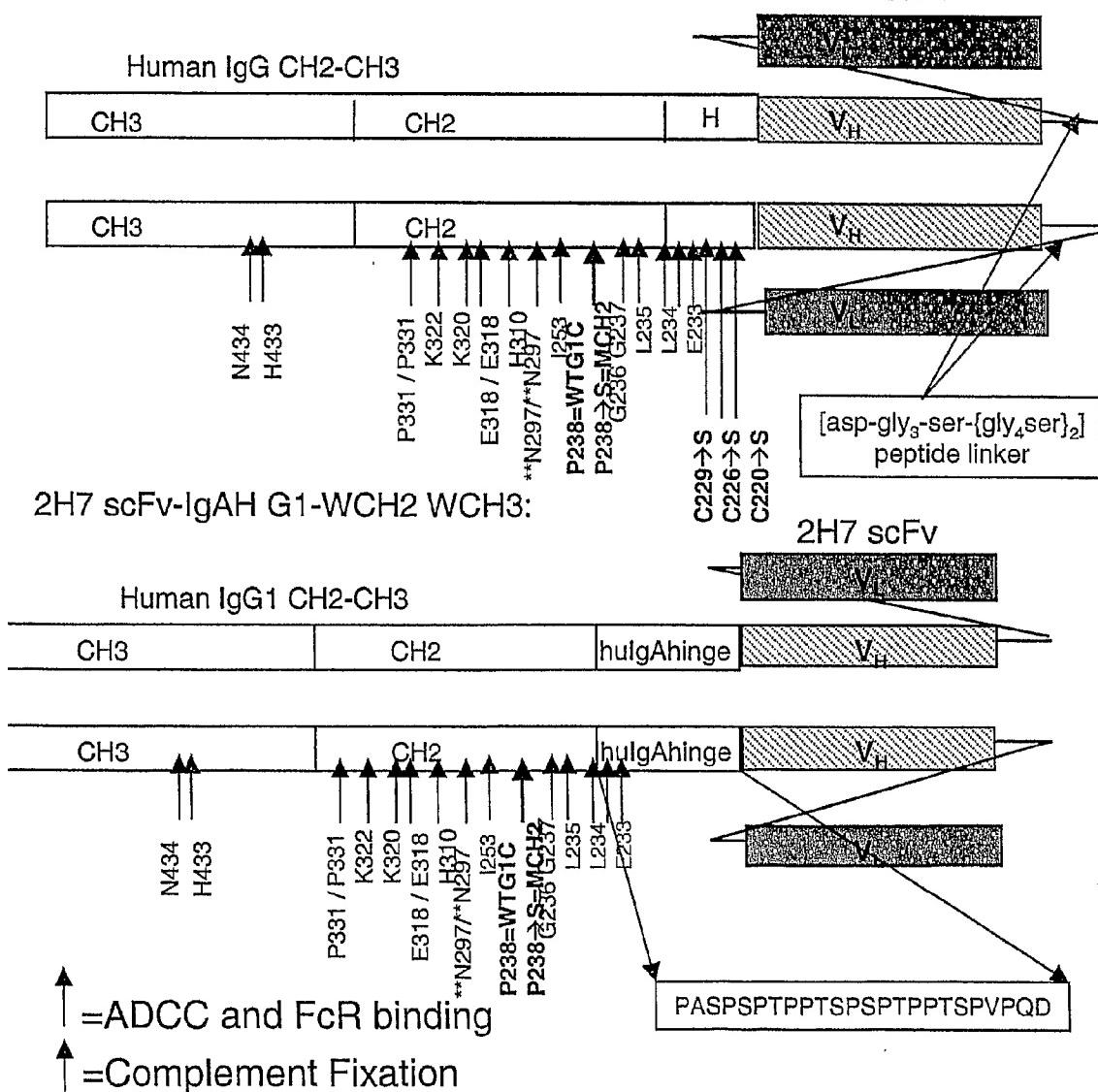


Fig. 12

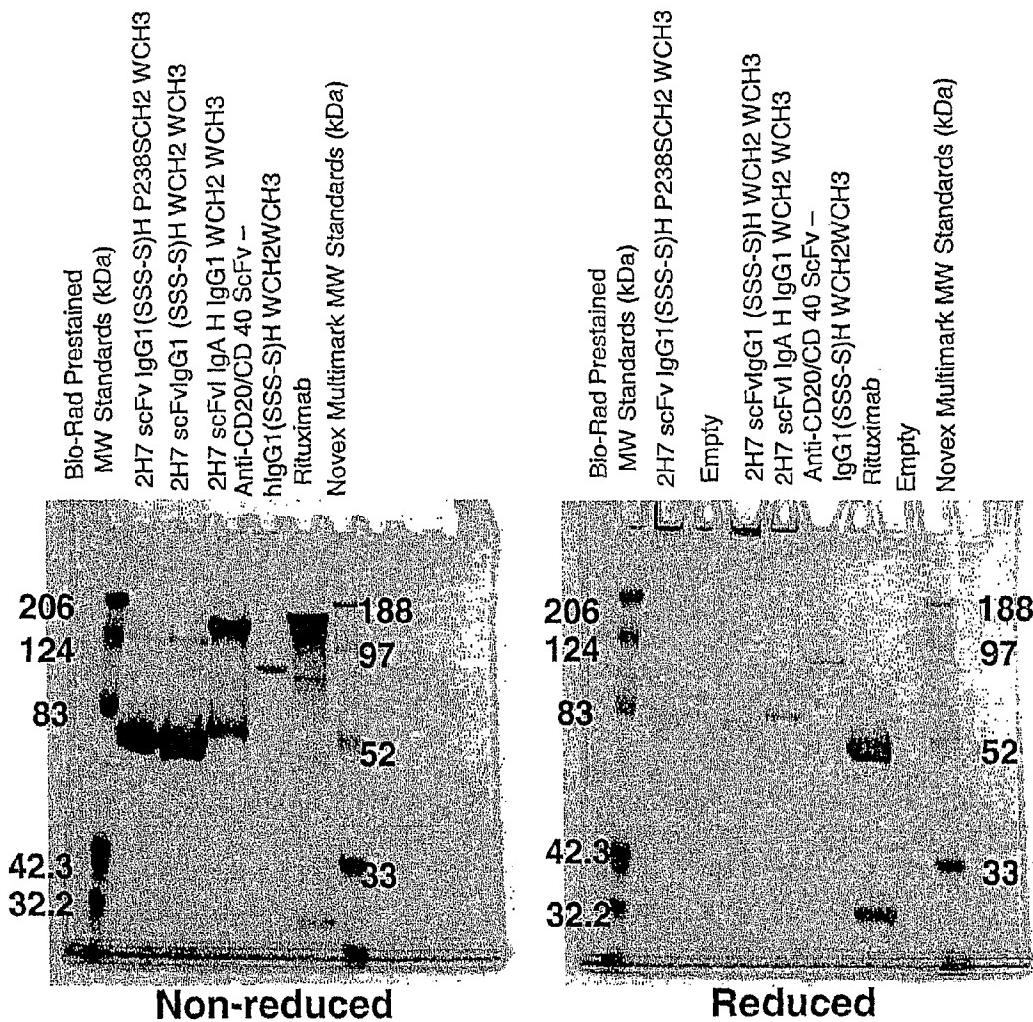


Figure 12: SDS-PAGE Analysis of CytoxB Derivatives. Purified fusion protein derivatives of CytoxB-scFvIg molecules and Rituximab were resuspended SDS sample buffer, boiled, loaded onto 10% Novex Tris-Bis gels (Invitrogen, San Diego, CA) and subjected to nonreducing (left panel) or reducing (right panel) SDS-PAGE electrophoresis at 175 volts. Two different molecular weight markers, BioRad prestained markers, and Novex Multimark molecular weight markers were also loaded onto each gel and the approximate size in kDa of each marker band is indicated along each side of the photographed gels. Gels were stained in Coomassie Blue stain and photographed with a SONY Mavica Digital camera. The mutant hinge forms of 2H7 scFvIgG1 migrate at approximately 70 kDa under both nonreducing and reducing conditions, indicating that these molecules are monomeric rather than dimeric in structure. The IgA hinge form of 2H7scFvIg migrates at approximately 75 kDa under reducing conditions, but migrates predominately as a dimer of 140 kDa with a fraction of the protein migrating at 75 kDa under nonreducing conditions. Under nonreducing conditions, rituximab migrates as a diffuse band of between 150 and 200 kDa. The heavy and light chains resolve into separate bands of approximately 32 and 50 kDa when rituximab is reduced and subjected to SDS-PAGE.

Fig. 13

## ADCC Activity of CytoxB (2H7 scFvIg) Constructs.

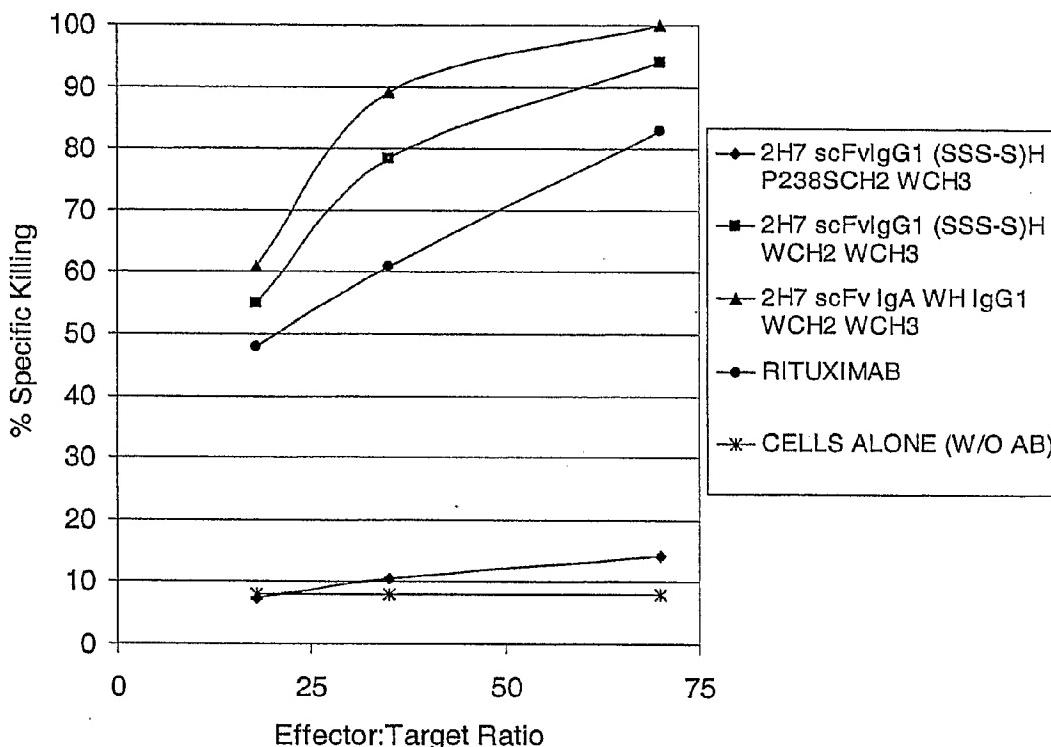


Figure 13: ADCC Activity of CytoxB Derivatives Compared to Rituximab. ADCC activity of CytoxB Derivatives or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with  $^{51}\text{Cr}$  and aliquoted at a cell density of  $5 \times 10^4$  cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 mg/ml, and PBMC were added at  $9 \times 10^5$  cells /well (18:1),  $1.8 \times 10^6$  cells/well (35:1), or  $3.6 \times 10^6$  cells/well (70:1), in a final volume of 200  $\mu\text{l}$ . Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 ml culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

Fig. 14

## CDC of Cytox B (2H7 scFvIg) Constructs

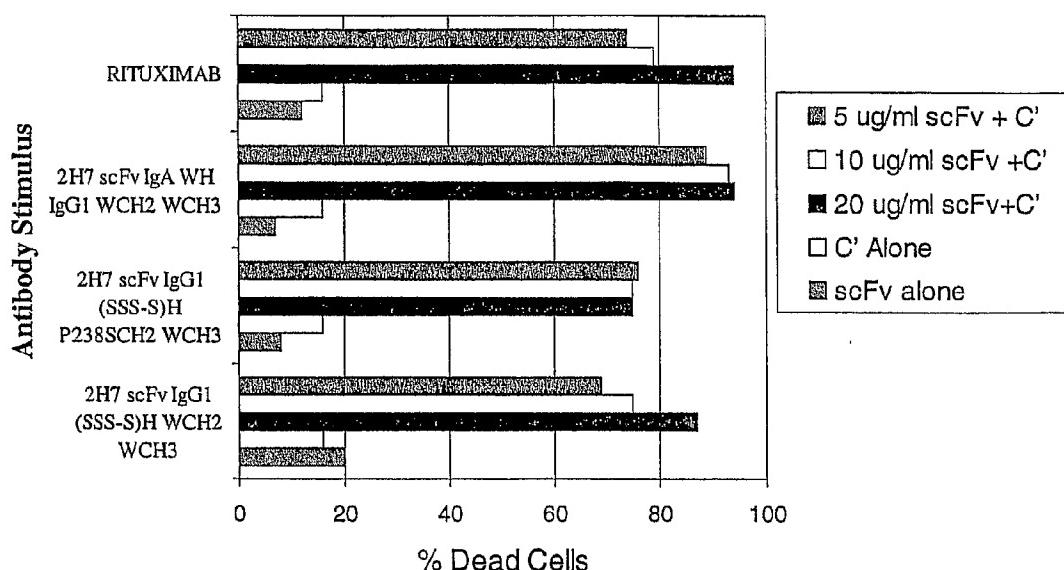


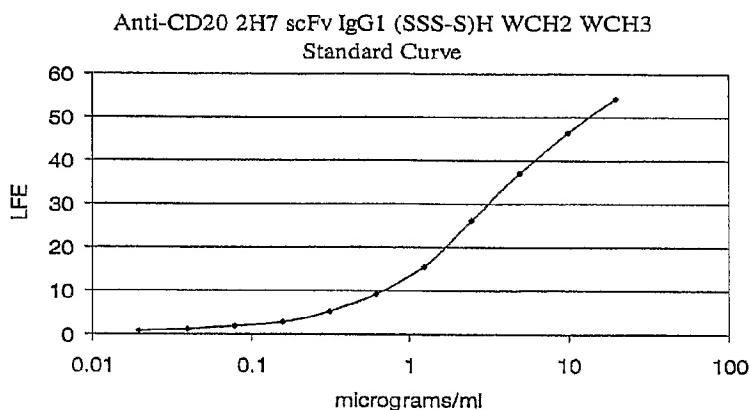
Figure 14: Complement Dependent Cytotoxicity (CDC) Activity of CytoxB Derivatives Compared to Rituximab. 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, and 2H7scFv IgA WH IgG1 WCH2 WCH3 derivatives and Rituximab were compared for their ability to mediate complement dependent cytotoxicity. Rabbit complement (Pel-Freez) was diluted 1:10 and added to BJAB cells along with dilutions of each antibody derivative (20  $\mu$ g/ml, 10  $\mu$ g/ml, and 5  $\mu$ g/ml). Controls were also included without addition of complement (C') or scFv derivative. Reactions were allowed to continue for 1 hour, and cells from each well were then stained with trypan blue and the cell viability counted using a hemacytometer. Data is graphed as % of dead cells/total cells counted for each condition assayed.

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Fig. 15

**2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3  
In Vivo Half Life**



Macaque A99314

| Day | Binding intensity (LFE)<br>@ 1:50 dilution of serum | estimated<br>concentration ( $\mu$ g/ml) |
|-----|-----------------------------------------------------|------------------------------------------|
| -7  | 0.213                                               | <0.1                                     |
| 0   | 0.227                                               | <0.1                                     |
| 1   | 7.79                                                | 25.1                                     |
| 3   | 5.51                                                | 15.6                                     |
| 7   | 3.37                                                | 9.4                                      |
| 8   | 11.33                                               | 41.7                                     |
| 10  | 5.45                                                | 15.4                                     |
| 14  | 0.27                                                | <0.1                                     |

Injection #1 → Day -7, 0, 1, 3, 7, 8, 10, 14

Injection #2 → Day 7, 8

Macaque F98081

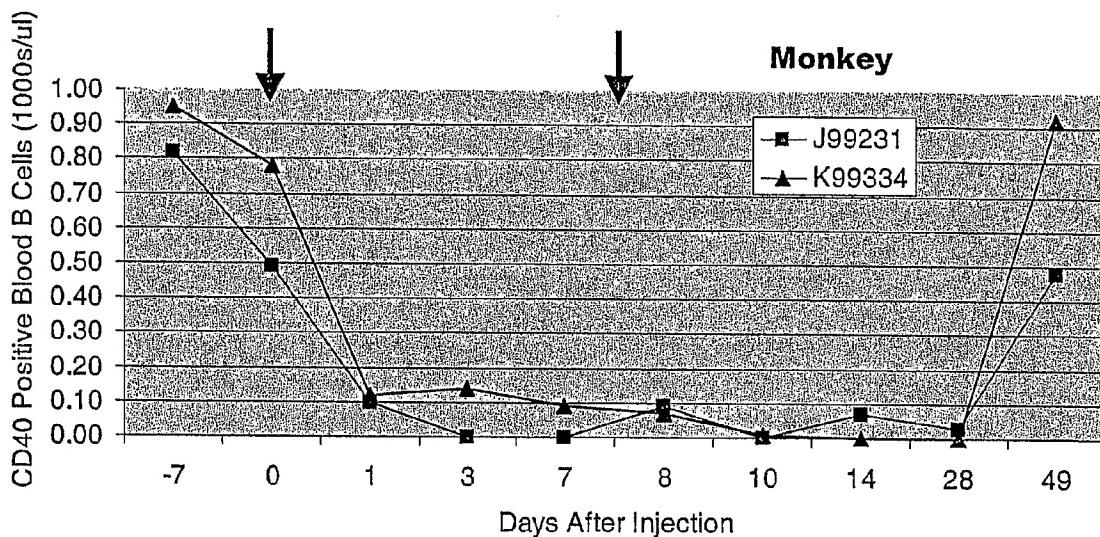
| Day | Binding intensity (LFE)<br>@ 1:50 dilution of serum | estimated<br>concentration ( $\mu$ g/ml) |
|-----|-----------------------------------------------------|------------------------------------------|
| -7  | 0.208                                               | <0.1                                     |
| 0   | 0.219                                               | <0.1                                     |
| 1   | 6.73                                                | 21.9                                     |
| 3   | 6.14                                                | 19.3                                     |
| 7   | 3.04                                                | 8.7                                      |
| 8   | 9.83                                                | 33.8                                     |
| 10  | 4.77                                                | 14.4                                     |
| 14  | 0.231                                               | <0.1                                     |

Injection #1 → Day -7, 0, 1, 3, 7, 8, 10, 14

Injection #2 → Day 7, 8

Fig. 16

B Cell Depletion in macaques mediated by Cytox B20  
(2H7 scFv IgG1 (SSS-S)H WCH2 WCH3) Construct

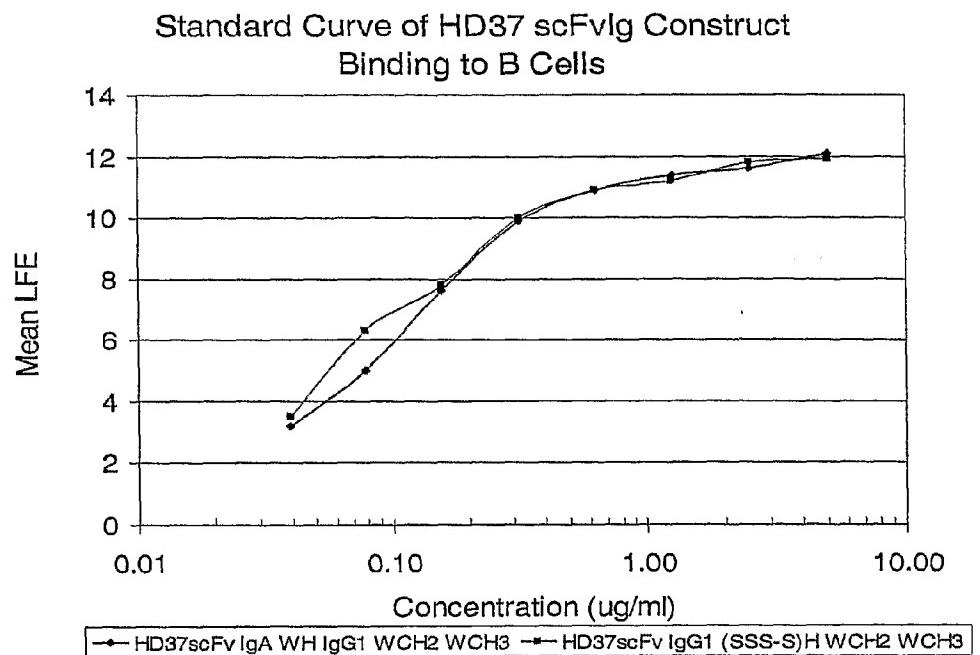


- CytoxB20 injections of 6mg/kg yields 3 week B-cell depletion
- 3-4 day half-life *in vivo*
- CD20 saturation in lymph node B-cells at d14
- No first dose effects
- No anti-chimeric antibody development

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Fig. 17

Production Levels of HD37 scFvIg Constructs  
by CHO Cell Lines

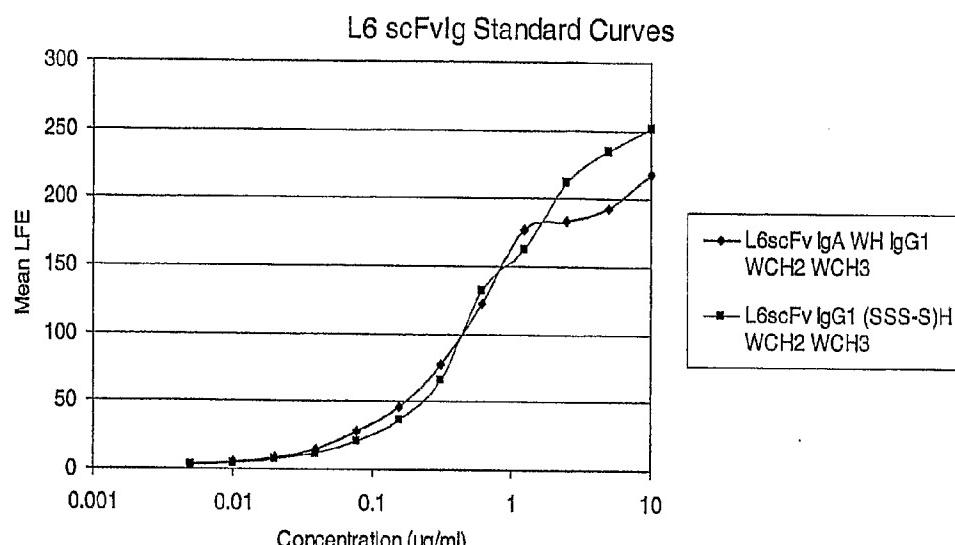
| Clone/Isolate           | Mean LFE at 1:100 | Estimated Concentration |
|-------------------------|-------------------|-------------------------|
| Bulk HD37 scFv          |                   |                         |
| IgA WH IgG1 WCH2 WCH3   | 11.2              | > 60 ug/ml              |
| 1B2                     | 10.4              | >50 ug/ml               |
| 6C5                     | 10.5              | >50 ug/ml               |
| 4B1                     | 8.6               | >40 ug/ml               |
| Bulk HD37 scFv          |                   |                         |
| IgG1 (SSS-S)H WCH2 WCH3 | 10.9              | > 50 ug/ml              |
| 2G8                     | 10.6              | > 50 ug/ml              |
| 3F3                     | 8.3               | >40 ug/ml               |
| 3D9                     | 11.1              | > 60 ug/ml              |

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Fig. 18

### Production of L6 scFvIg constructs by CHO Cells



| Construct                                               | Mean LFE 1:20 | Estimated Concentration |
|---------------------------------------------------------|---------------|-------------------------|
| L6scFv IgA WH<br>IgG1 WCH2 WCH3<br>unamplified CHO sup  | 51.1          | 6.25 ug/ml              |
| L6scFv IgG1(SSS-S)H<br>WCH2 WCH3<br>unamplified CHO sup | 23.0          | 3.2 ug/ml               |

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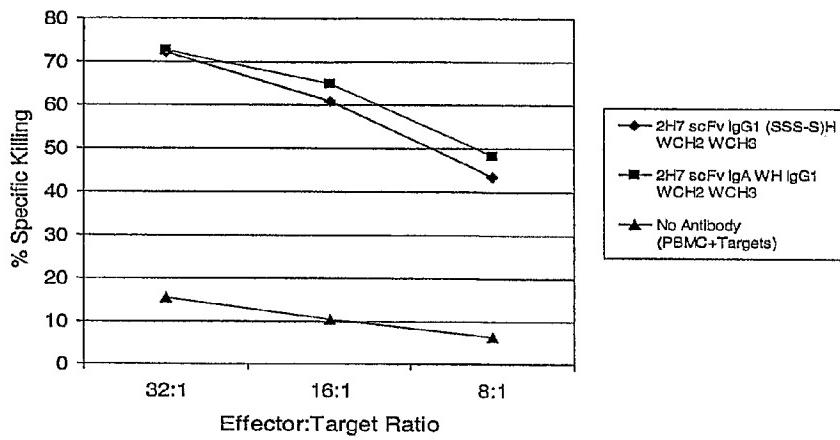
PCT/US2003/041600

**Fig. 19**

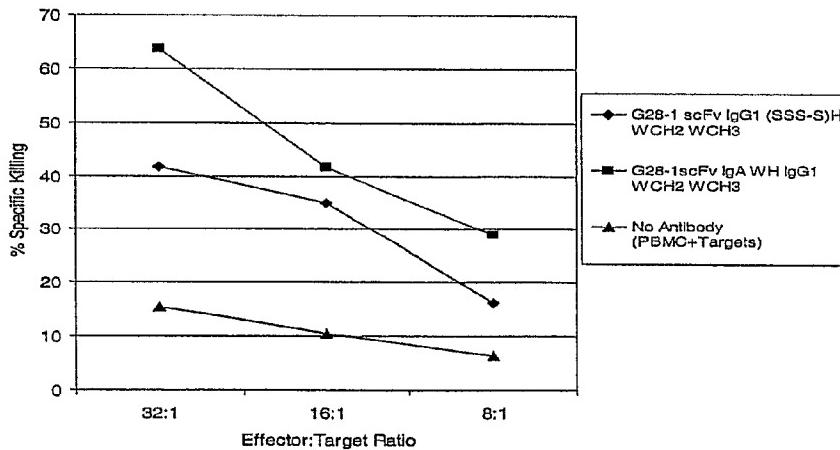
## ADCC Activity of 2H7 scFvIg, G28-1 scFvIg, and HD37 scFvIg Constructs

### ADCC Activity of scFvs Targeted to B Cell Antigens

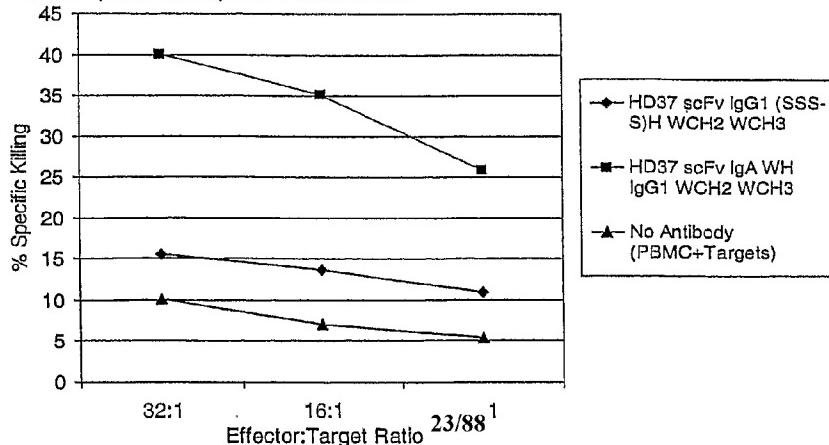
#### A. 2H7 (anti-CD20) scFv constructs



#### B. G28-1 (anti-CD37) scFv constructs



#### C. HD37 (anti-CD19) scFv constructs

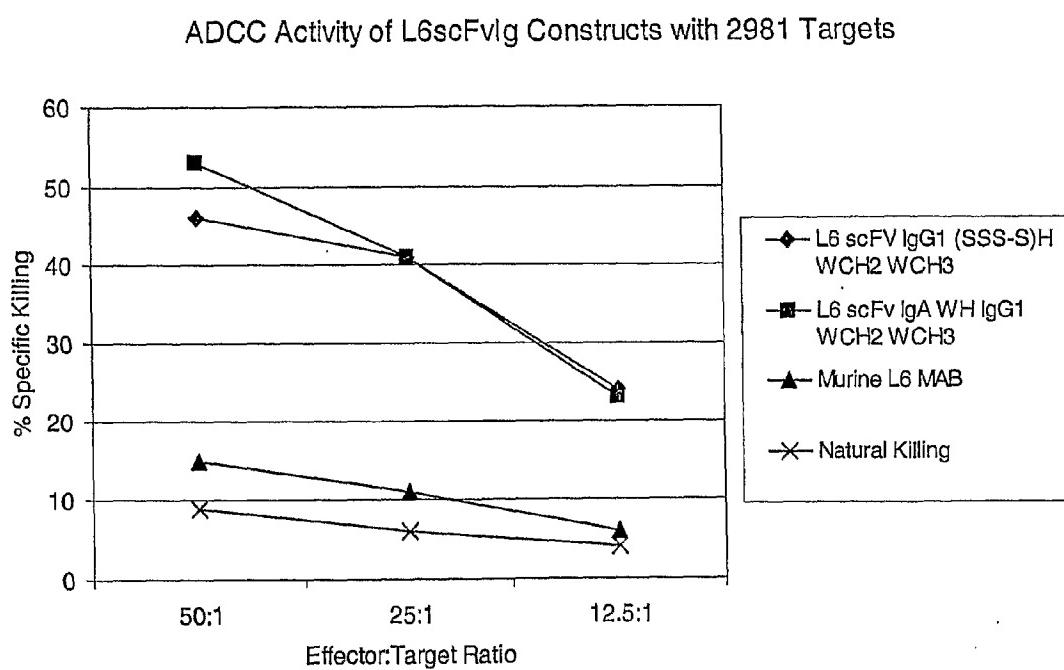


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Fig. 20

### ADCC Activity of L6 scFvIg Constructs

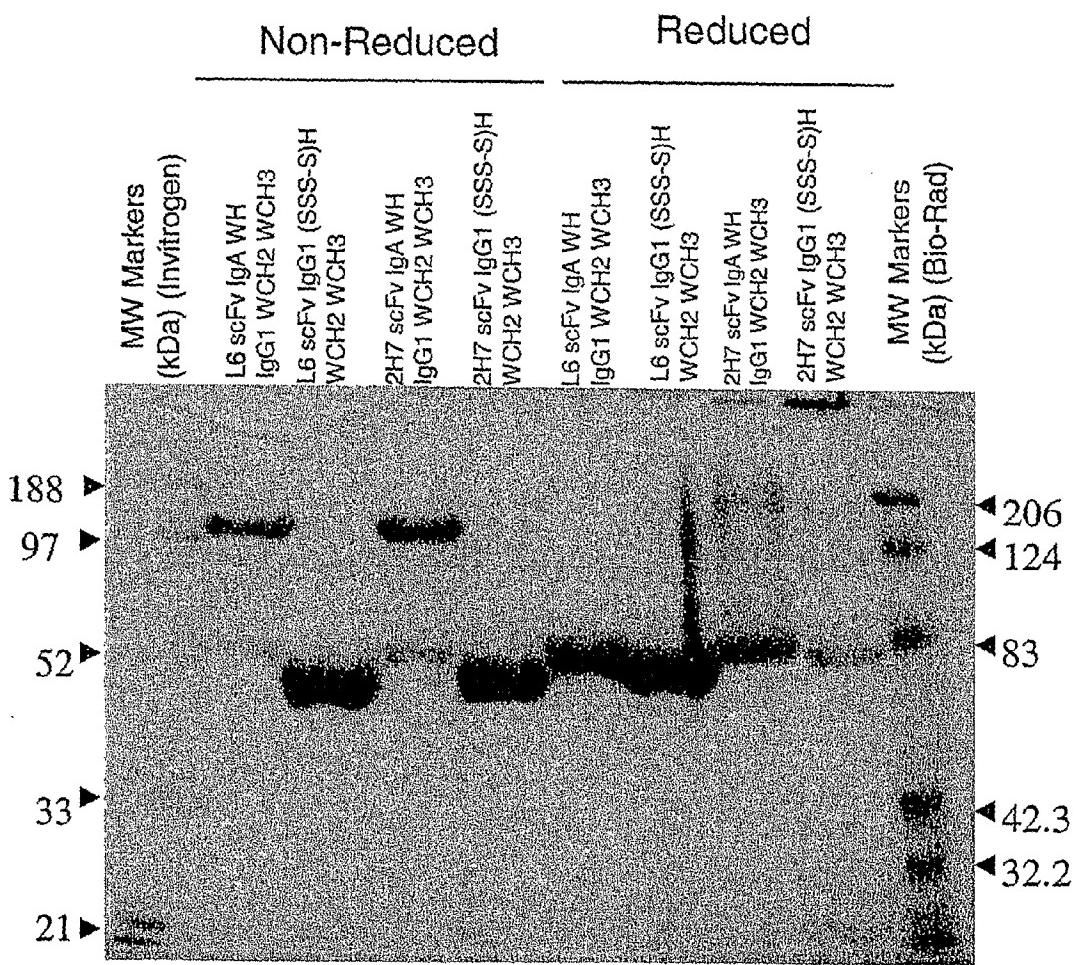


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Fig. 21

SDS-PAGE Analysis of L6 and 2H7 scFvIg Fusion Proteins.

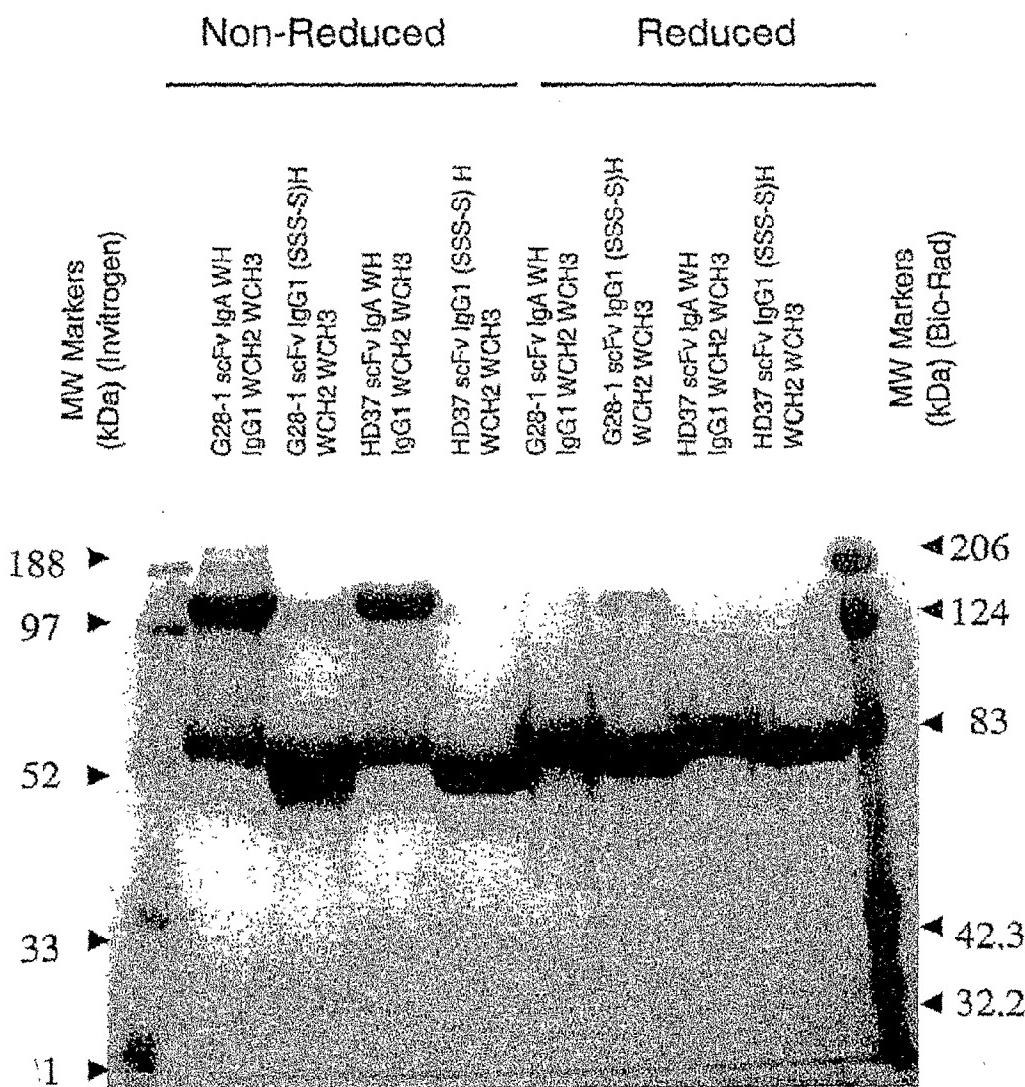


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Fig. 22

SDS-PAGE Analysis of G28-1 and HD37 scFvIg Constructs.



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Fig. 23

Sequence alignment of human and llama Fc regions.

HINGE

CH2 →

|          |                            |                                                      |
|----------|----------------------------|------------------------------------------------------|
| an IgG1: | DQEPKSCDKT-----HTCPC       | PAPELLGGPSVFLFPPKPKDTLMI                             |
| ma IgG2: | DQEPKTPKPQPQPQPNPTTESKCPKO | SRTPEVTCVVVDVSHEDPEVKFNWYVDG                         |
| ma IgG1: | --EPHGG-----CTCPQC         | PAPELLGGPSVIFPPKPKDVLISIGRPEVTCVVVDVGQEDPEVSPNWFIDG  |
| ma IgG3: | --AHHSEDPT-----SKCPKO      | PAPELPGGPSVFVFPKPKDVLISIGRPEVTCVVVDVGKEDPEVNWFIDG    |
|          |                            | PGPELLGGPTVIFPPKAKDVLISITRKPEVTCLWWTTWKKTLRSSSSWSVDD |

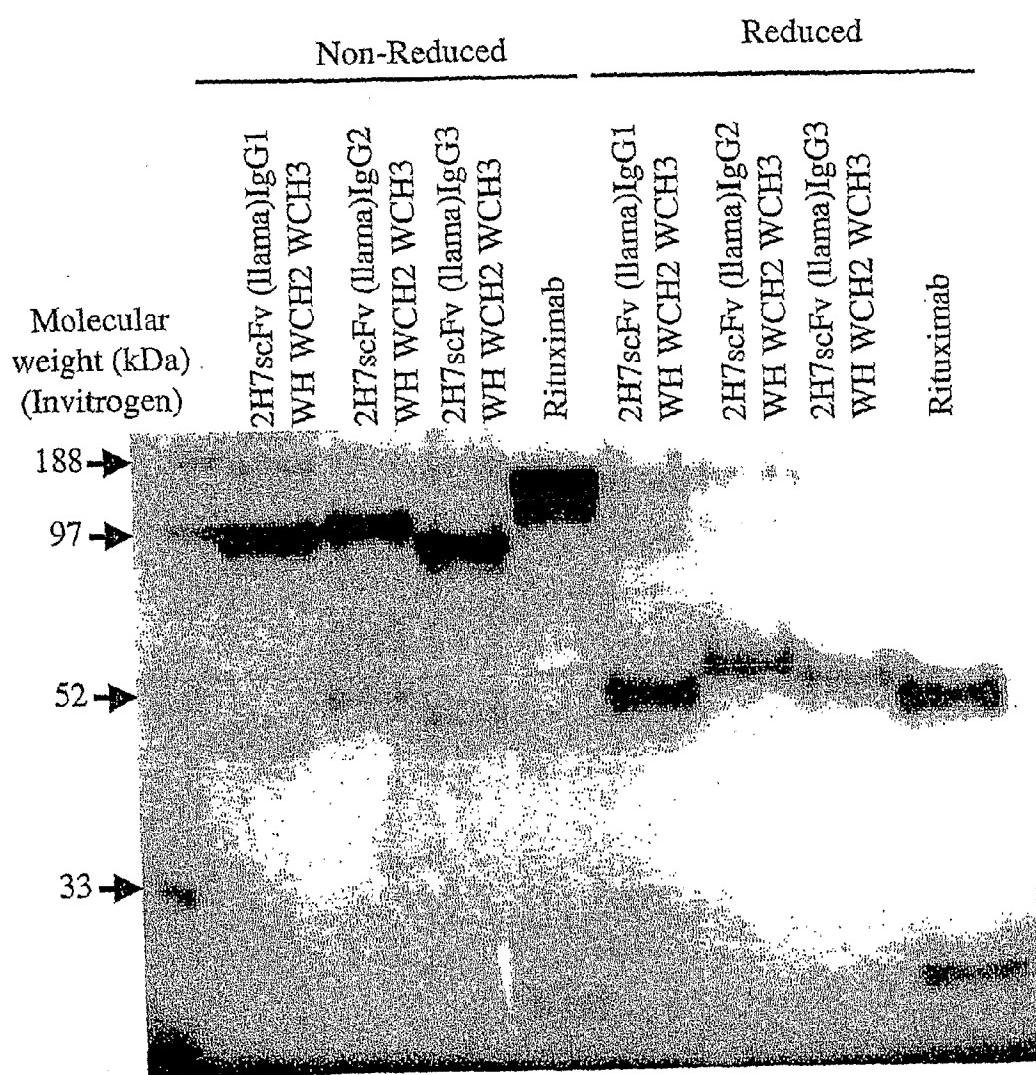
VEVHNAAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPVYTLPPSRDELTQNQVSILTAEVRANTRPKEEQFNSTYRVVSVLPIQHQDWLGTKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTVEVRTANTKPKKEEQFNSTYRVVSVLPIQHQDWLGTKEFKCKVNNKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTEVHTAETKPKKEEQFNSTYRVVSVLPIQHQDWLGTKEFKCKVNNKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVT

CLVKGFYPSDIAVEWESNGQOPEN--NYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVHEALHNHYTQKSLSLSPGKCLVKGFYPPDINVEWQRNGQPESEGTYATTPPQLDNDGTYFLXSKXSVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGKCLVKGFYPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSRLSUVGKNTWQRGETLTGVVVMHEALHNHYTQKSITQSSGKCLVKGFYPPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHYTQKSITQSSGK

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Fig. 24

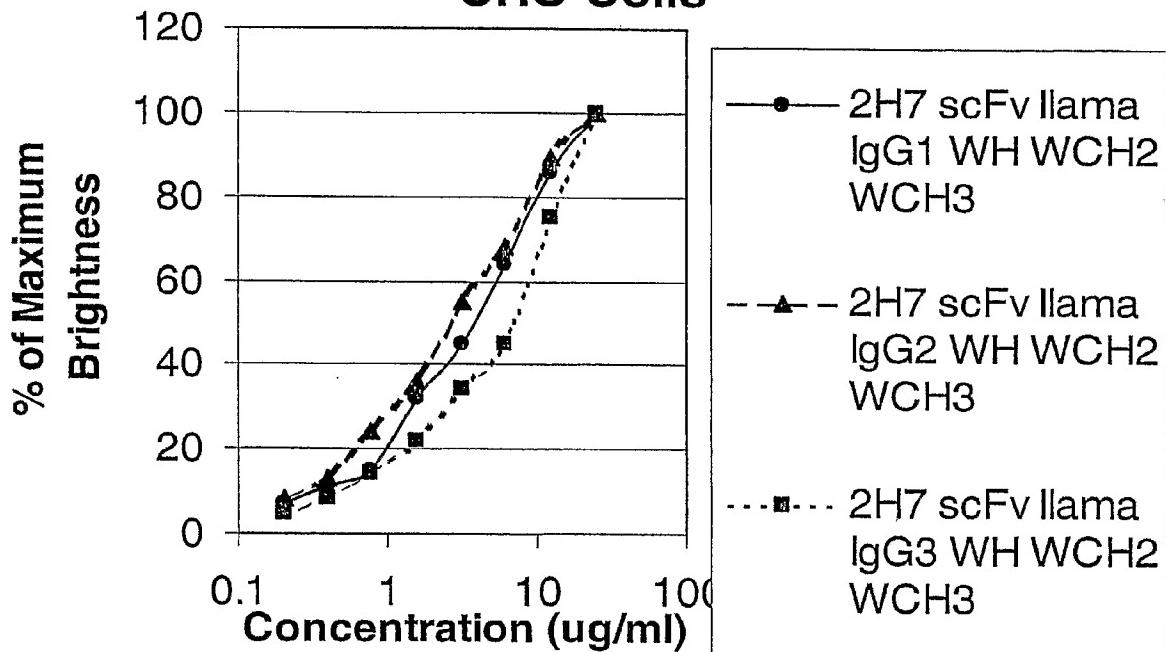


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Fig. 25

### Llama Tails Binding Assay with CD20 CHO Cells



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Fig. 26

2H7 scFv Ig Llama Tails binding Assay with CD20 CHO Cells

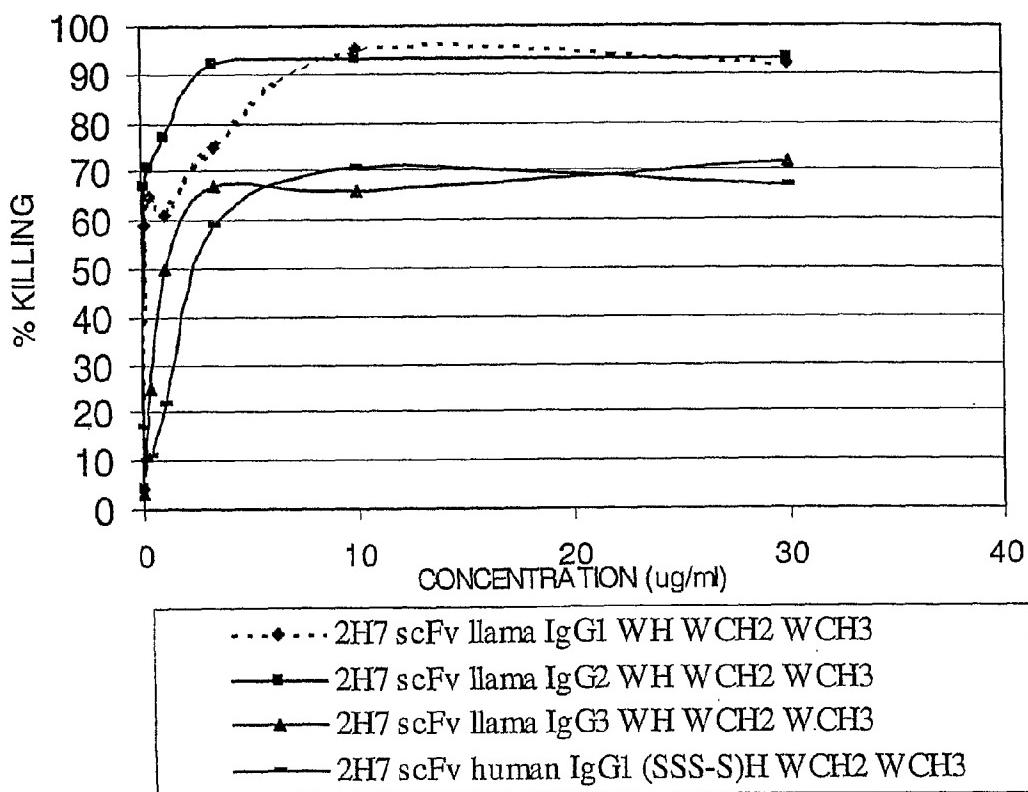
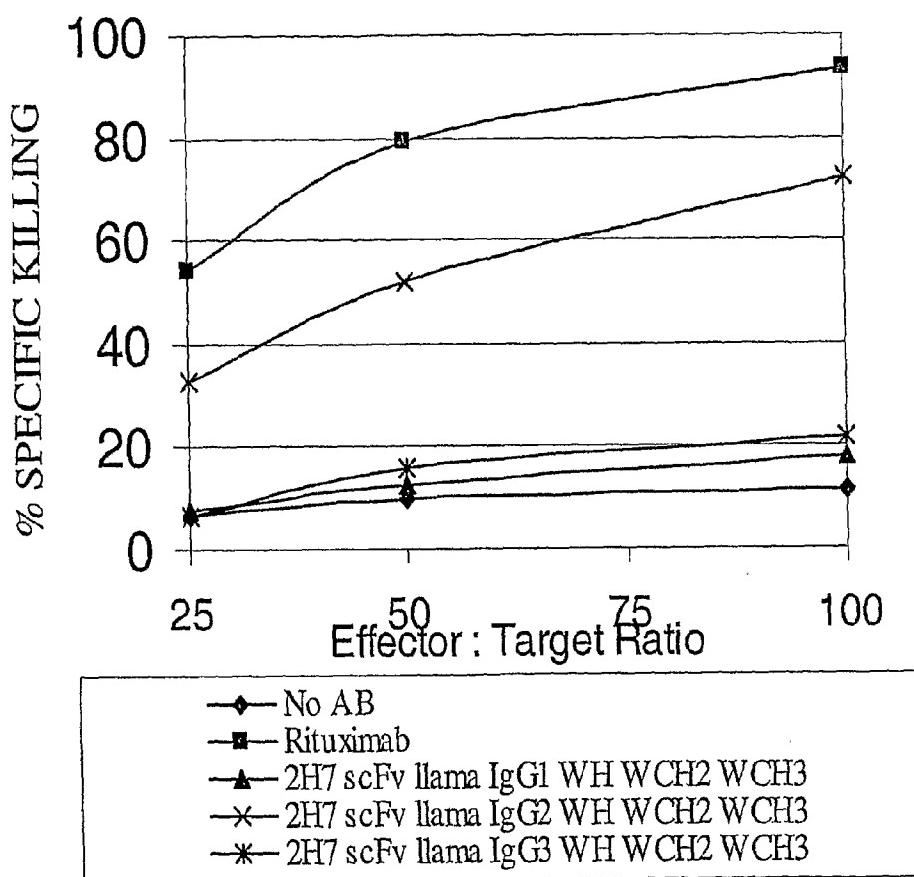


Fig. 27

ADCC Assay with BJAB Targets  
and Human PBMC Effectors

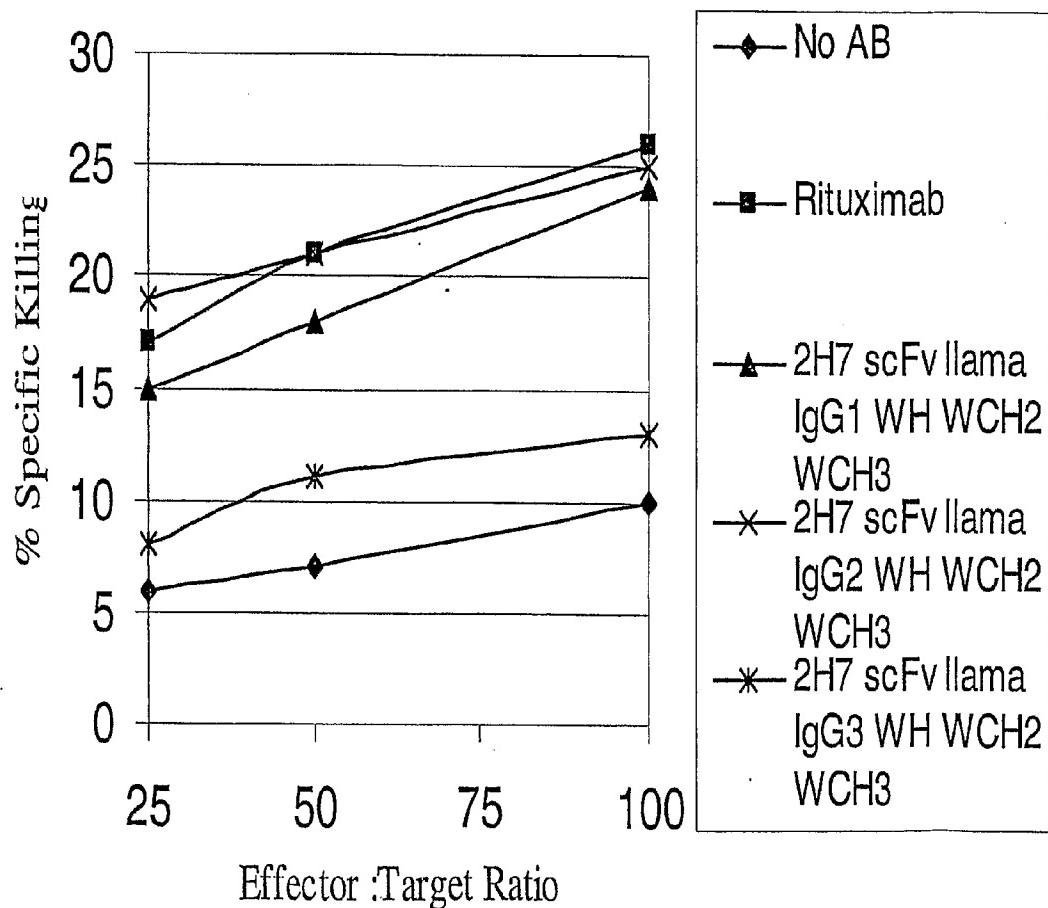


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Fig. 28

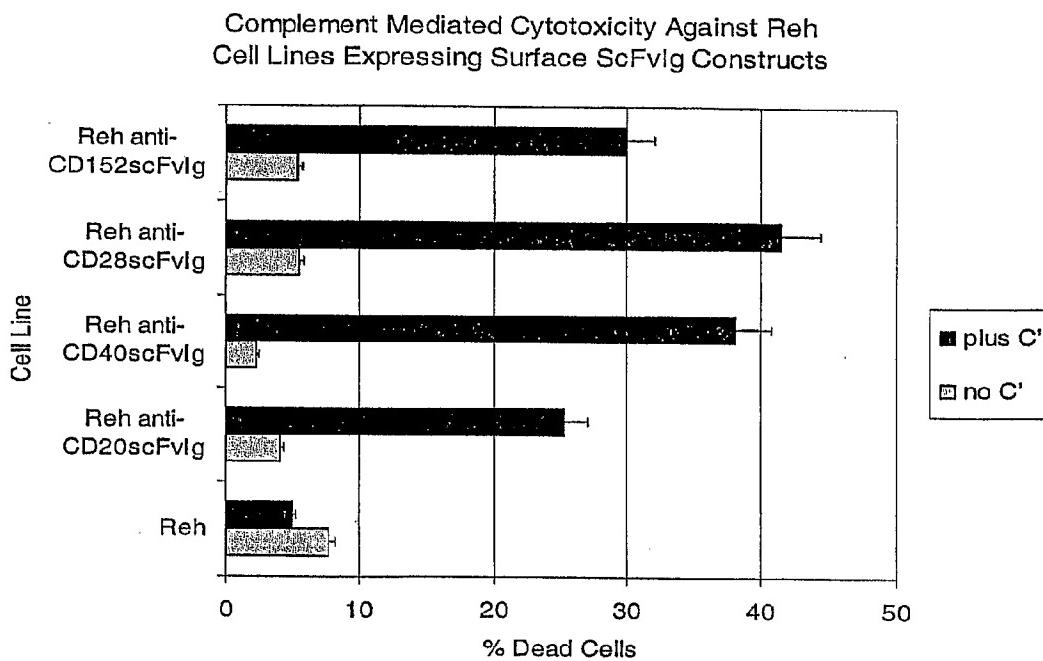
ADCC Assay with BJAB Cells  
And Llama PBMC Effectors



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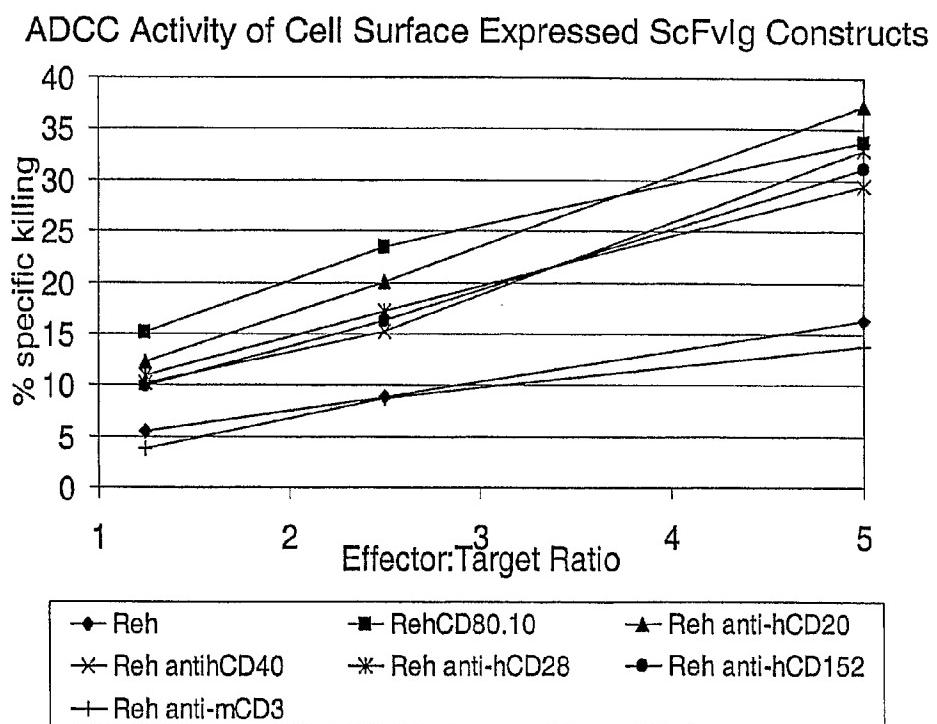
Fig. 29



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Fig. 30



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Fig. 31

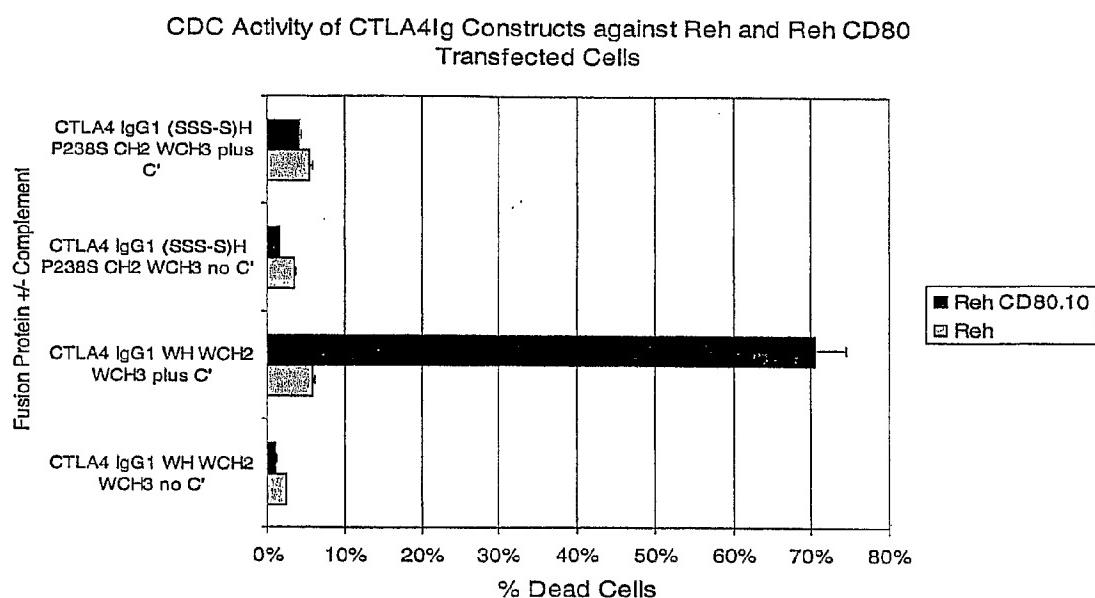
## Ig Constructs and Nomenclature:

| Name Identifier                        | Hinge Sequence               | CH2 Sequence                     | CH3 Sequence                                |
|----------------------------------------|------------------------------|----------------------------------|---------------------------------------------|
| hIgG1 (CCC-P)H<br>WCH2 WCH3            | IgG1 WT Hinge<br>(CCC-P)     | Wild Type CH2                    | Wild Type CH3                               |
| hIgG1 (SSS-S)H<br>WCH2 WCH3            | IgG1 Mutant Hinge<br>(SSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| VH L11S<br>hIgG1 (SSS-S)H<br>WCH2 WCH3 | IgG1 Mutant Hinge<br>(SSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SSC-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(SSC-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SCS-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(SCS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (CSS-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(CSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SSS-S)H<br>P238S CH2 WCH3        | IgG1 Mutant Hinge<br>(SSS-S) | Mutant CH2 (IgG1)<br>Pro→Ser 238 | Wild type CH3 (IgG1)                        |
| IgA WH hIgG1<br>WCH2 WCH3              | IgA Hinge                    | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgA WH IgA<br>WCH2 WCH3                | IgA Hinge                    | Wild type CH2 (IgA)              | Wild type CH3 (IgA)                         |
| IgA WH IgA<br>WCH2 T4CH3               | IgA Hinge                    | Wild type CH2 (IgA)              | Truncated CH3 (IgA)<br>Missing 4 aa at COOH |

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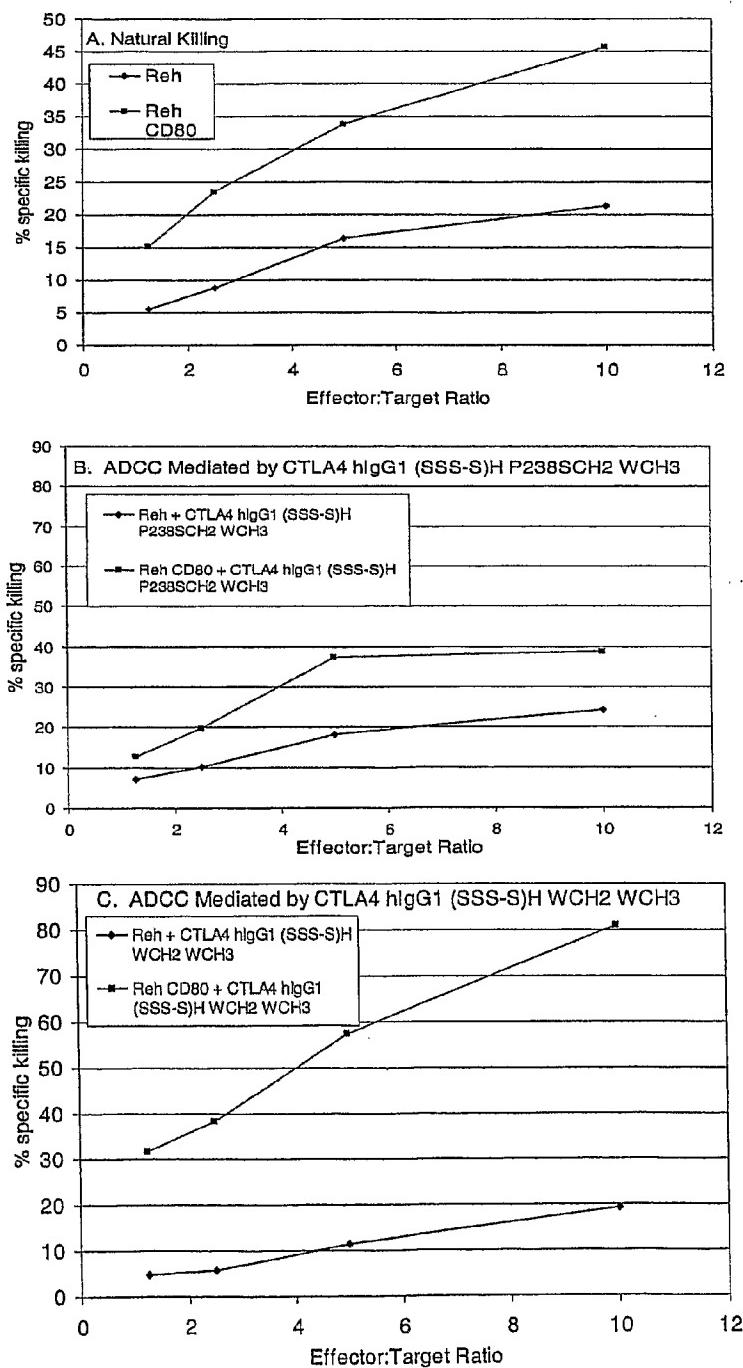
Fig. 32



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Fig. 33



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Fig. 34

Binding of 2H7 scFvIg Constructs  
with Alternative Tails to CD20 CHO Cells

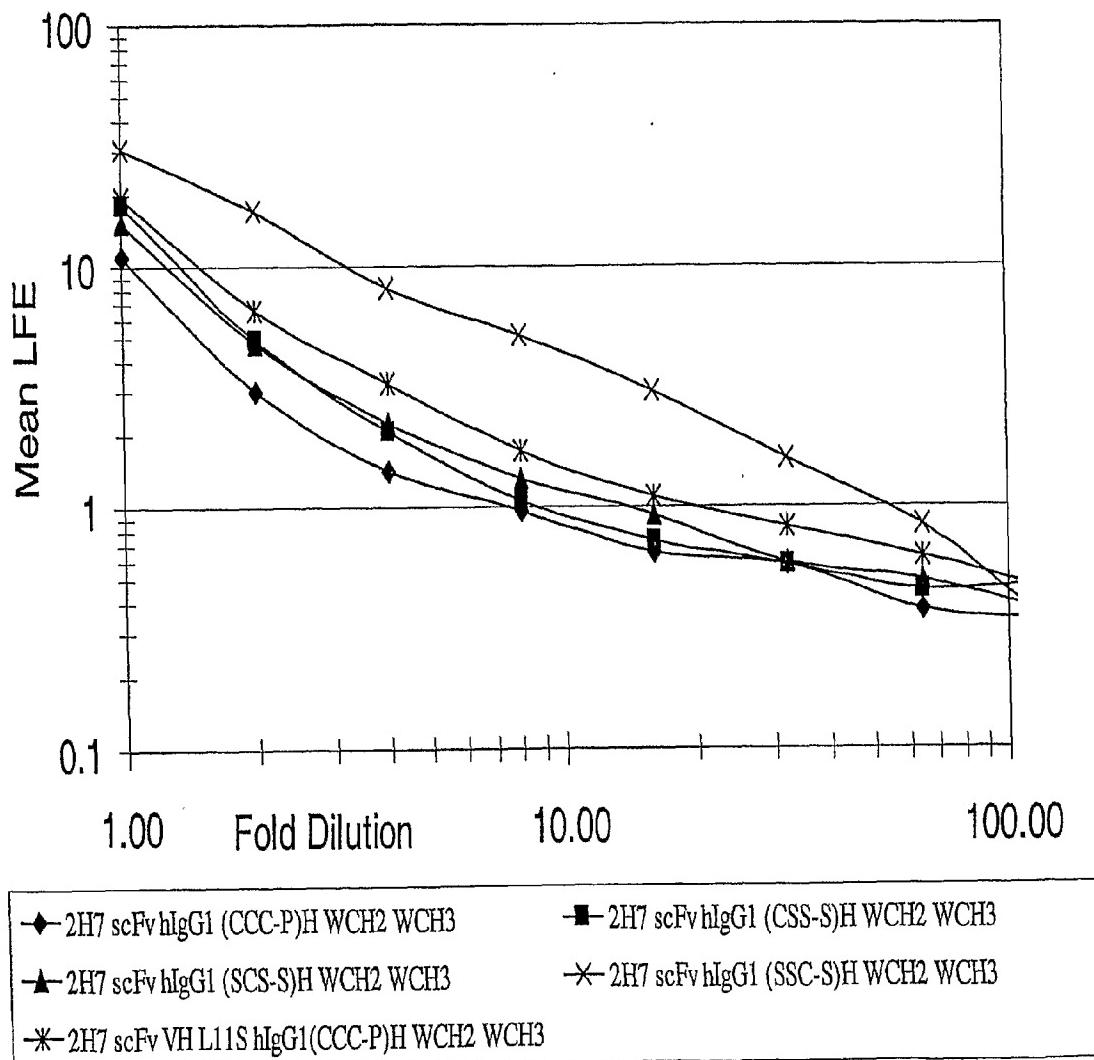
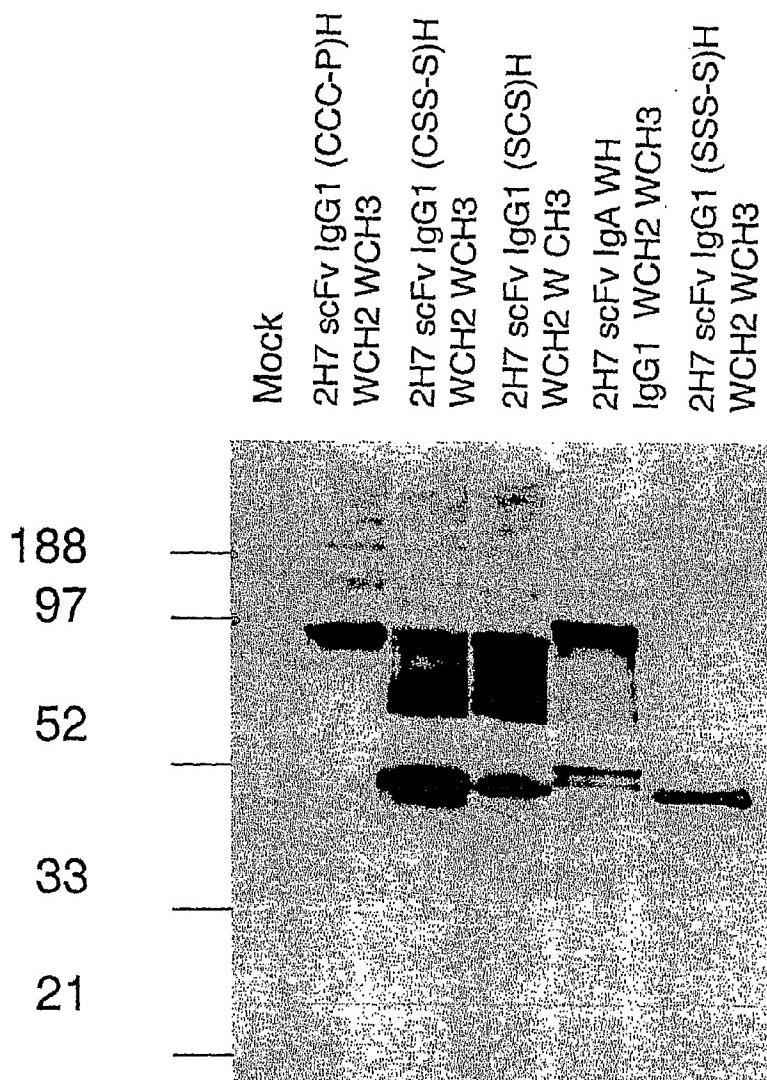


Fig. 35

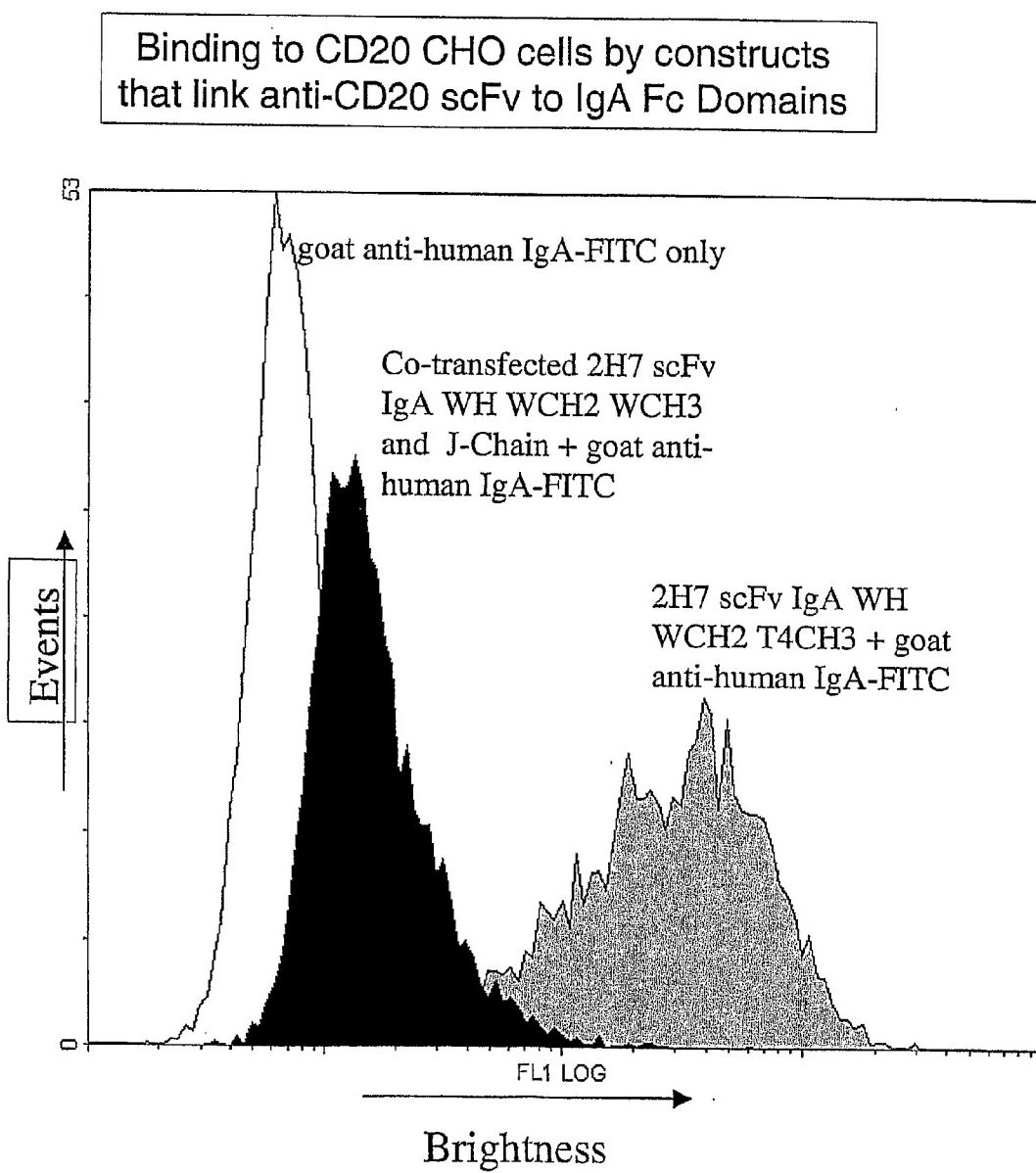
Immunoblot Analysis of protein immunoprecipitates  
from COS transfections of 2H7 scFvIg Constructs



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Fig. 36

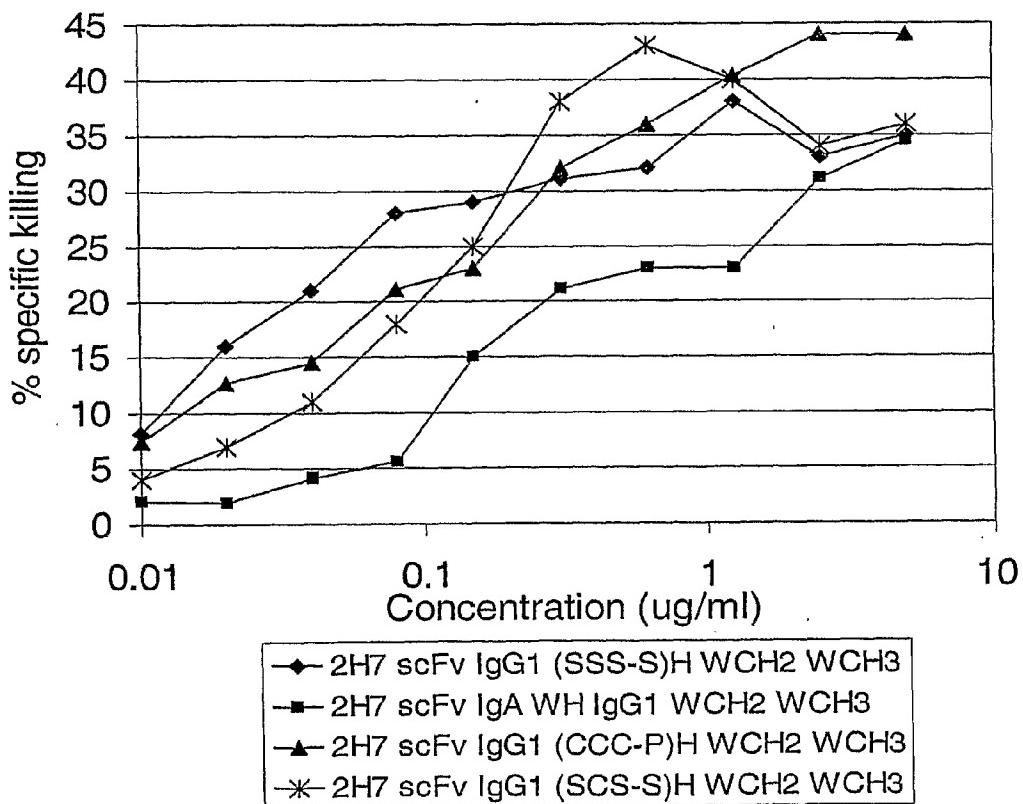


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Fig. 37

Titration of CD20 specific scFvIg Constructs  
for ADCC Activity Using Whole Blood Effectors

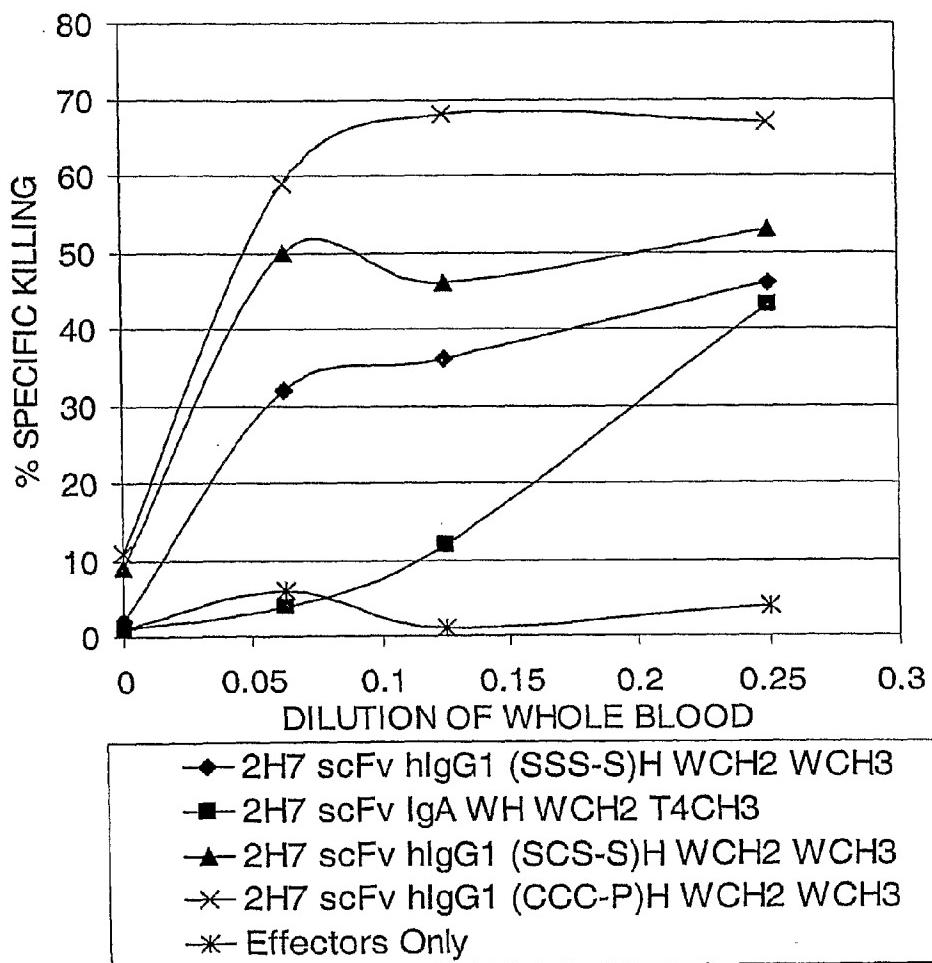


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Fig. 38

ADCC Assay of anti-CD20 constructs with alternative tails  
(Whole Blood Effectors / BJAB Targets)

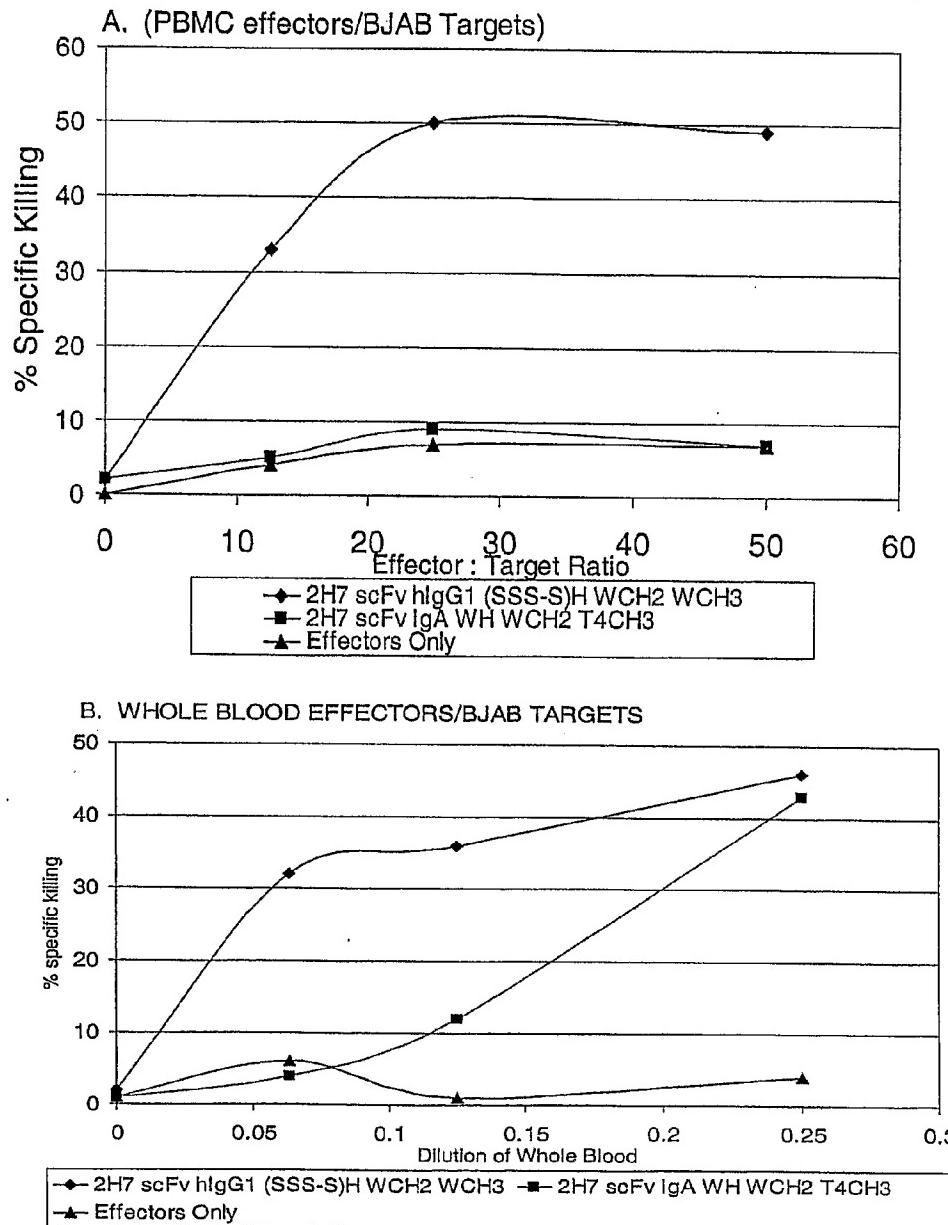


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Fig. 39

ADCC Assay of Anti-CD20 scFvIg Constructs  
Using Different Effector Populations Against BJAB Targets

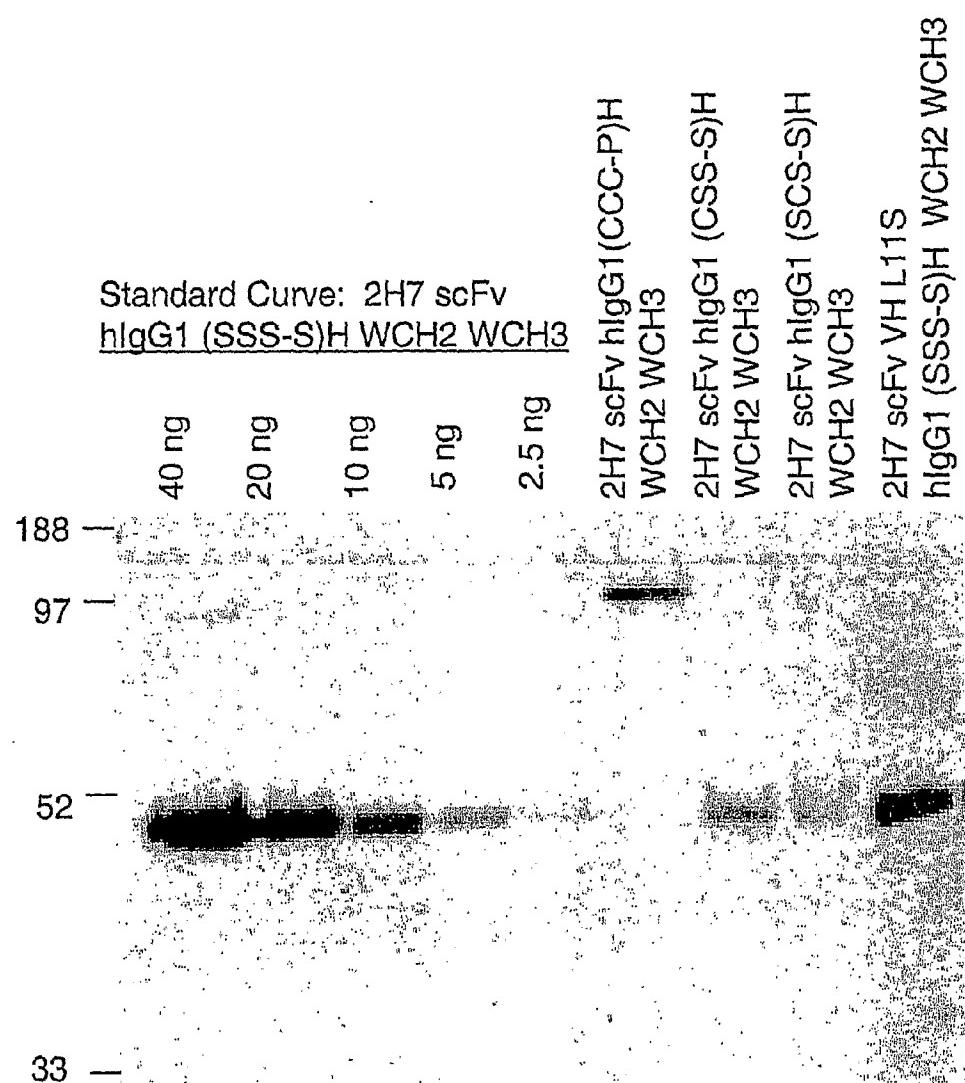


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Fig. 40

Immunoblot of 2H7 scFv Ig constructs from COS Transfections (1 µl/well) compared to a Concentration Standard

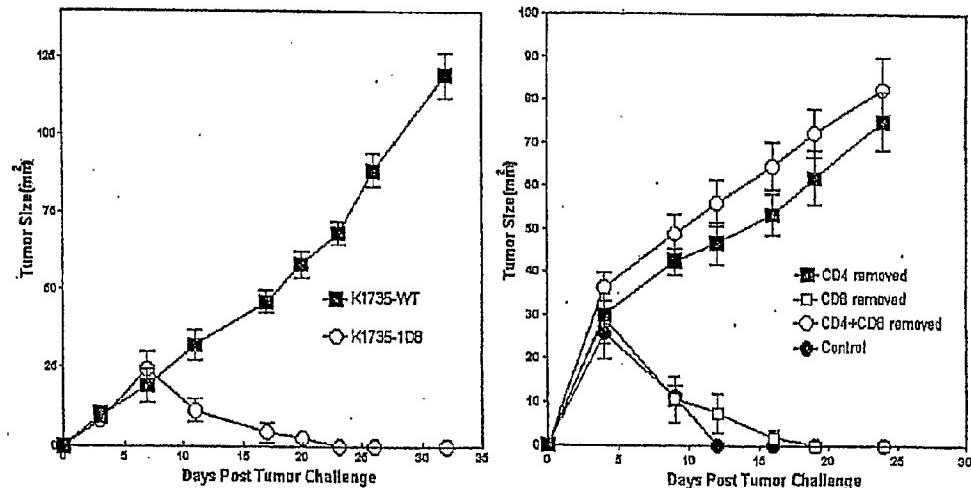
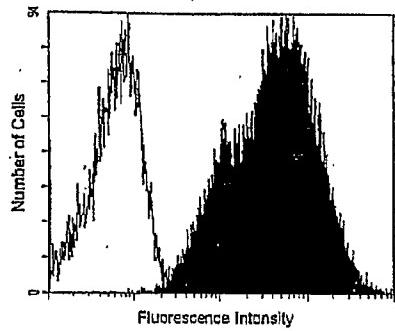


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## Figures 41A, 41B and 41C

A.



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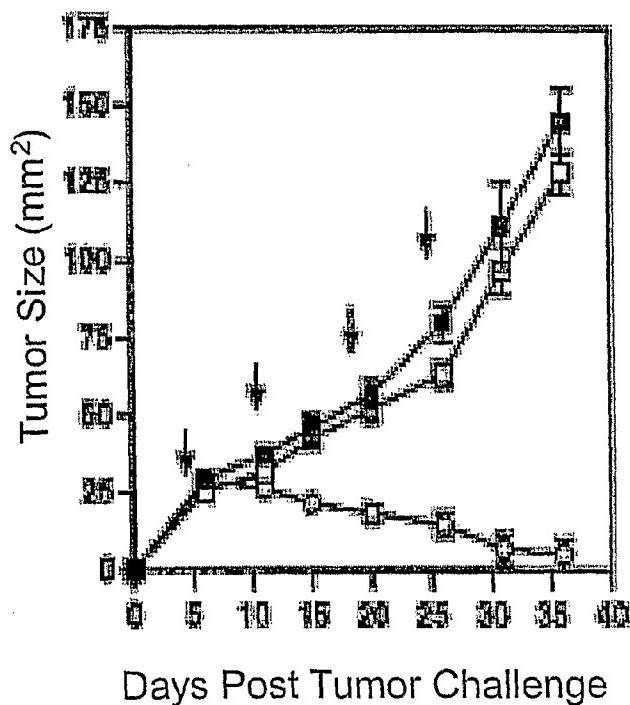
B.

C.

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Fig. 42

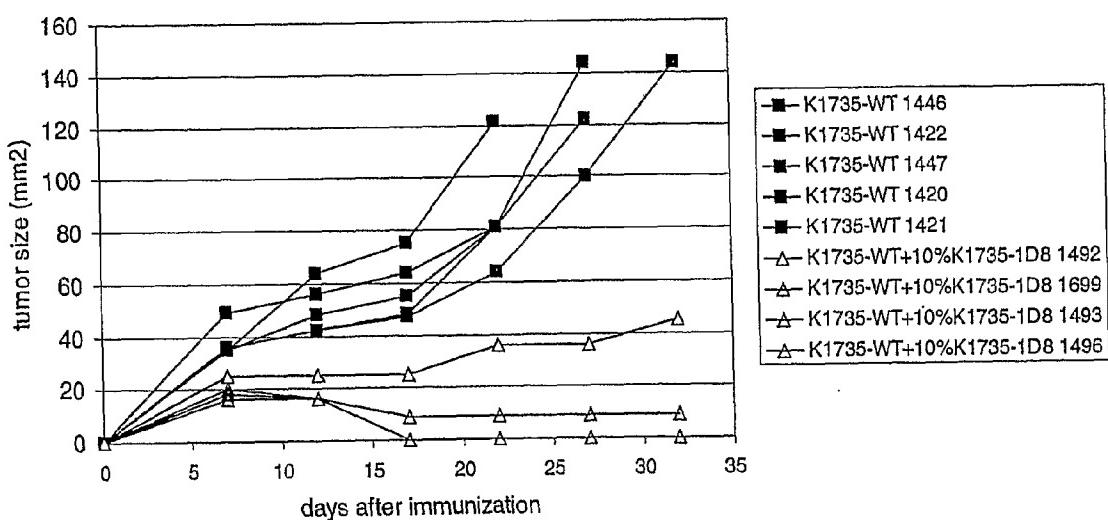


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Fig. 43

Mixtures of K1735-WT and K1735-1D8 transfected tumor lines inhibit tumor outgrowth in C3H mice

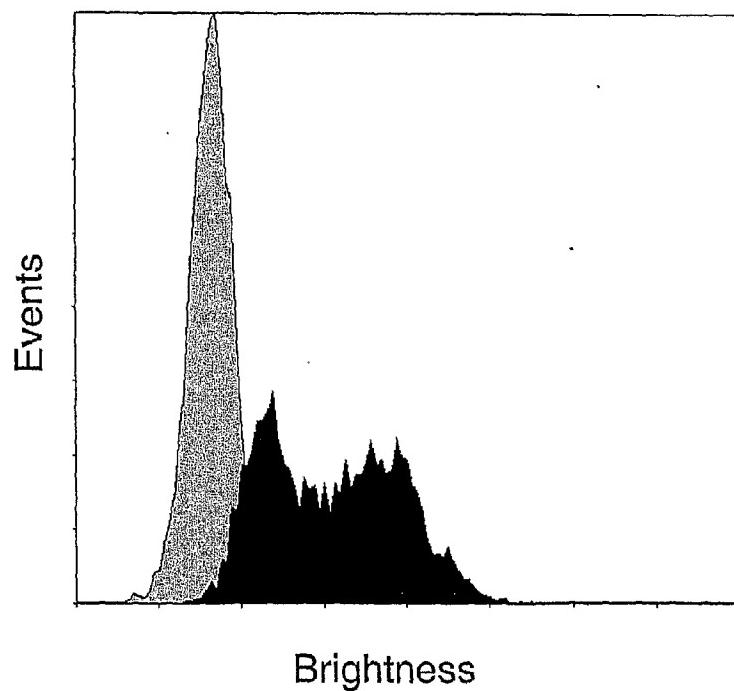


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Fig. 44

Expression of anti-mouse CD137 (1D8) scFv-hIgG1 (SSS-S)H P238SCH2 WCH3  
On the surface of panned Ag104-1D8 Transfected Tumor Cells

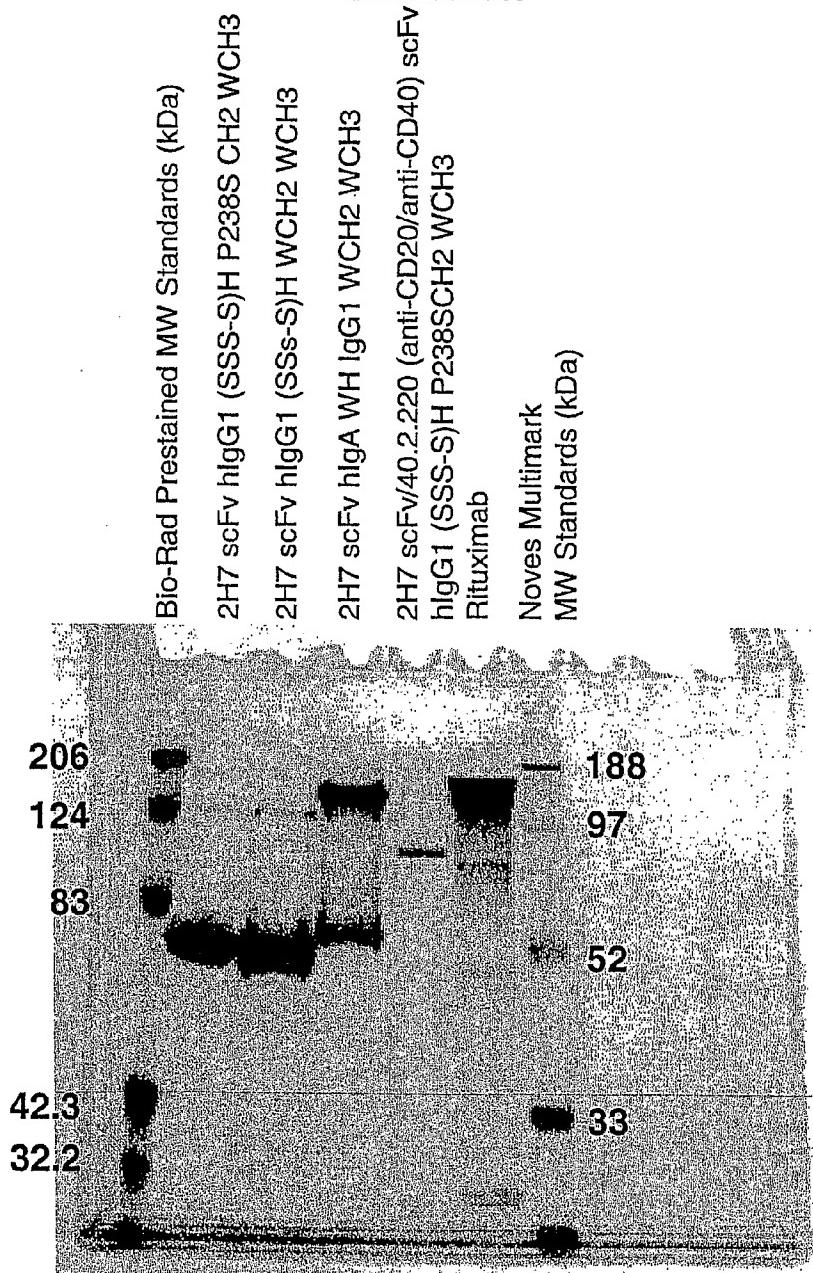


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Fig. 45

Coomassie Stained SDS-PAGE Gel of 2H7 scFv Ig Constructs

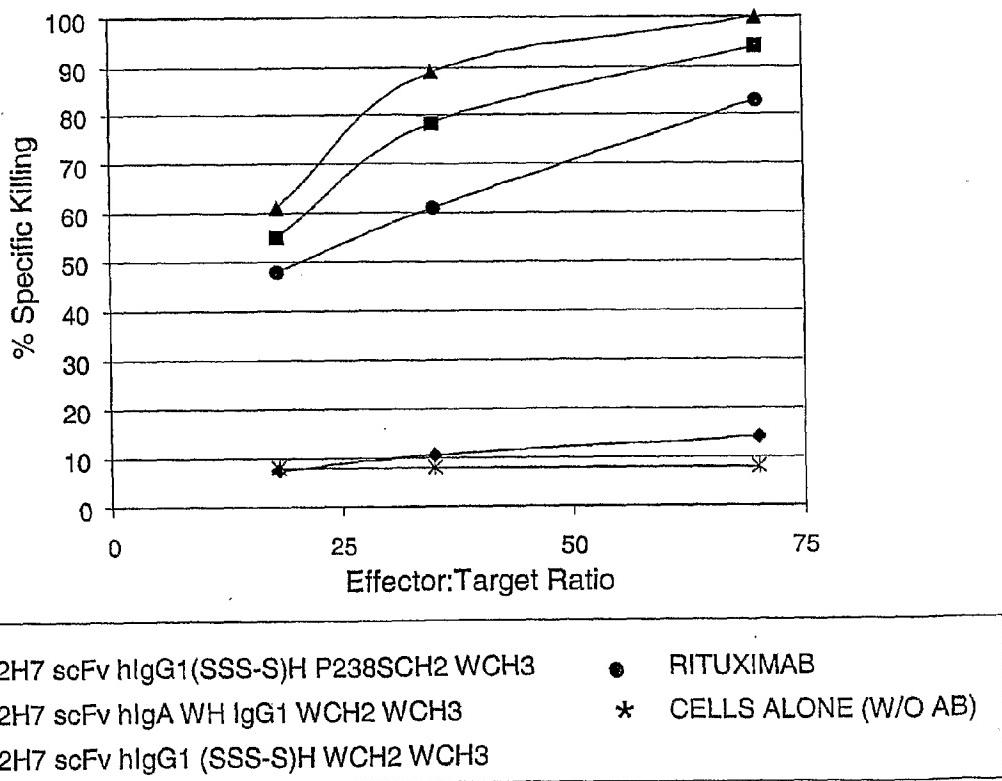


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Fig. 46

ADCC mediated by 2H7 scFvIg Constructs by human  
PBMC effector cells against Bjab targets



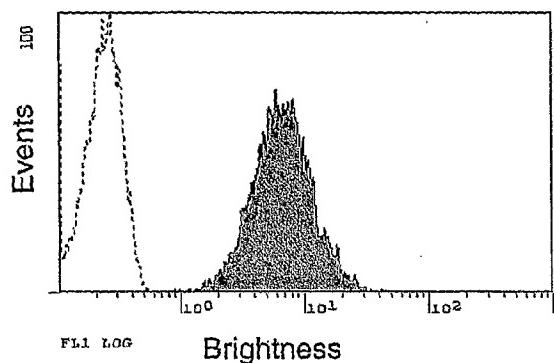
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Fig. 47

Cell surface expression of anti-human CD3 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT on Reh and T51 Cells.

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT



T51 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT:

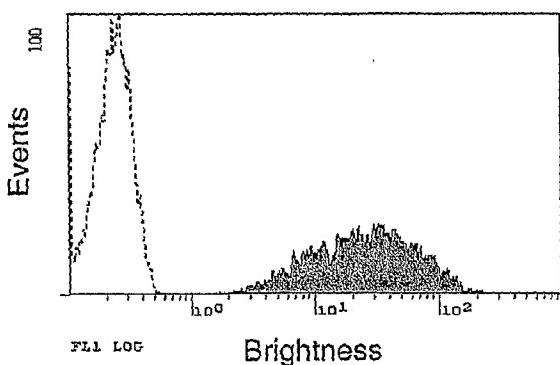
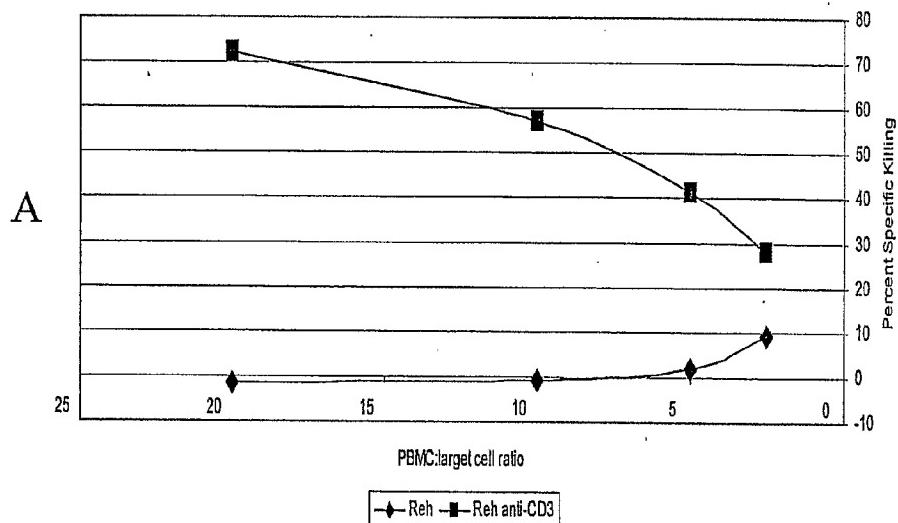
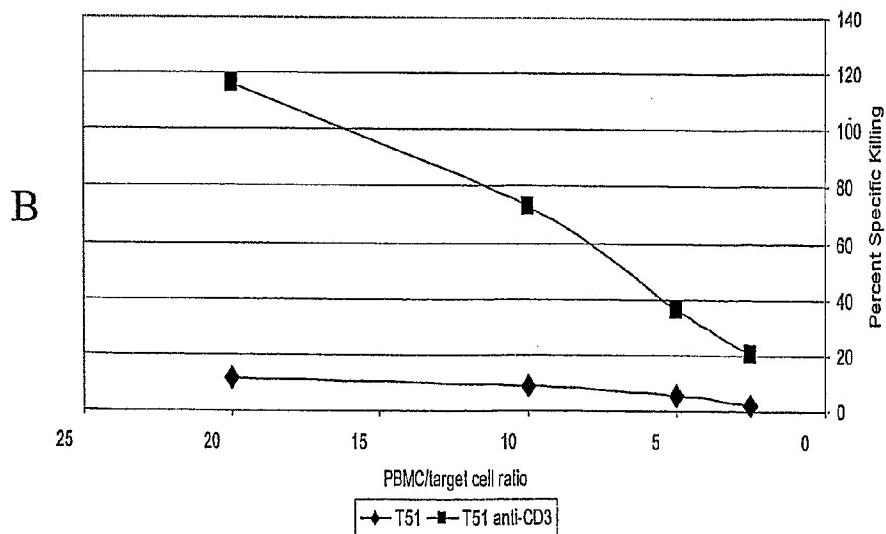


Figure 48.

Targeting of Cytotoxicity to Transfected Cell Lines  
by Surface expression of CD3 scFvIg  
Cytotoxic activity of resting PBMC towards transfected Reh cells



Cytotoxic activity of resting PBMC towards transfected T51 lymphoblastoid cells

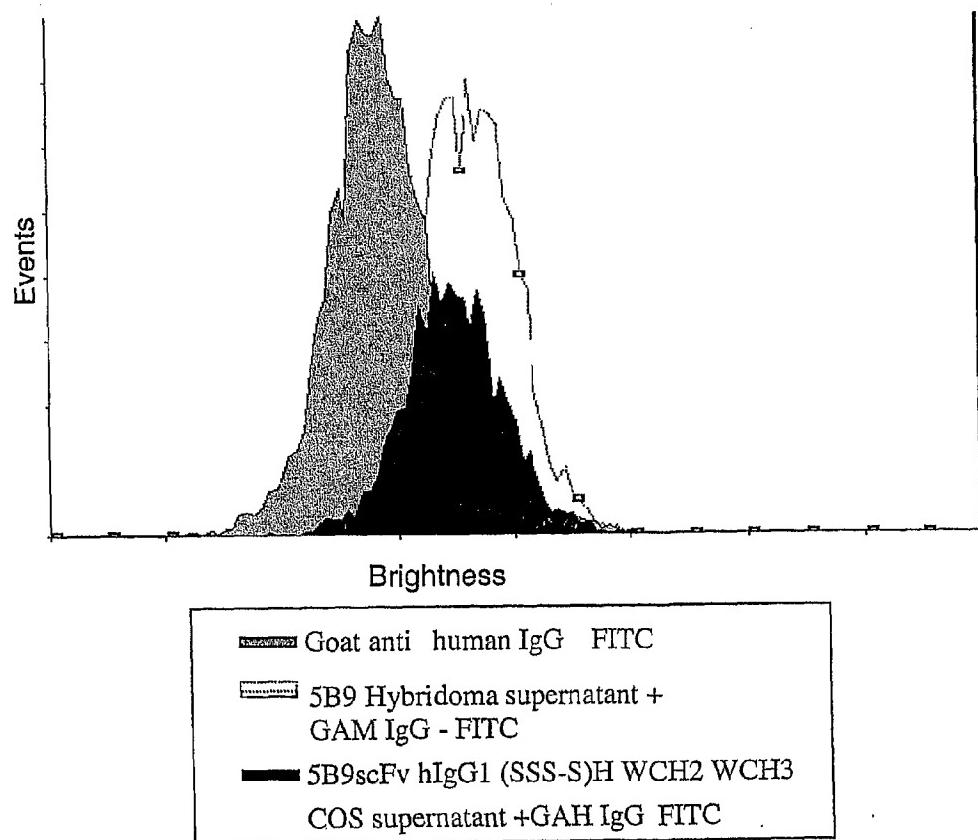


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Fig. 49

Binding of 5B9, a mouse anti-human CD137 scFv hIgG1 (SSS-S)H WCH2WCH3 to stimulated human PBMC



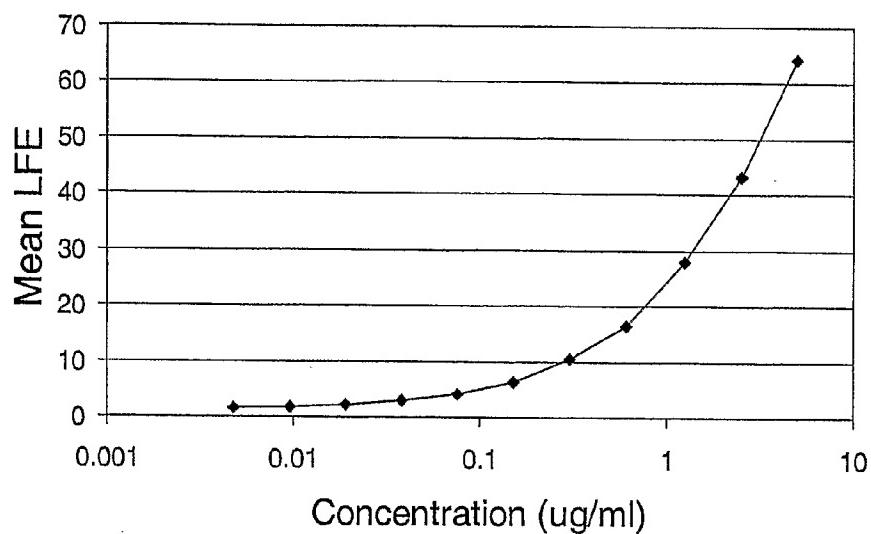
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Fig. 50

**Effect of V<sub>H</sub>L11S Mutation on CytoxB20  
2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 Protein Expression**

50A. Standard Curve: 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3



50B. CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve:

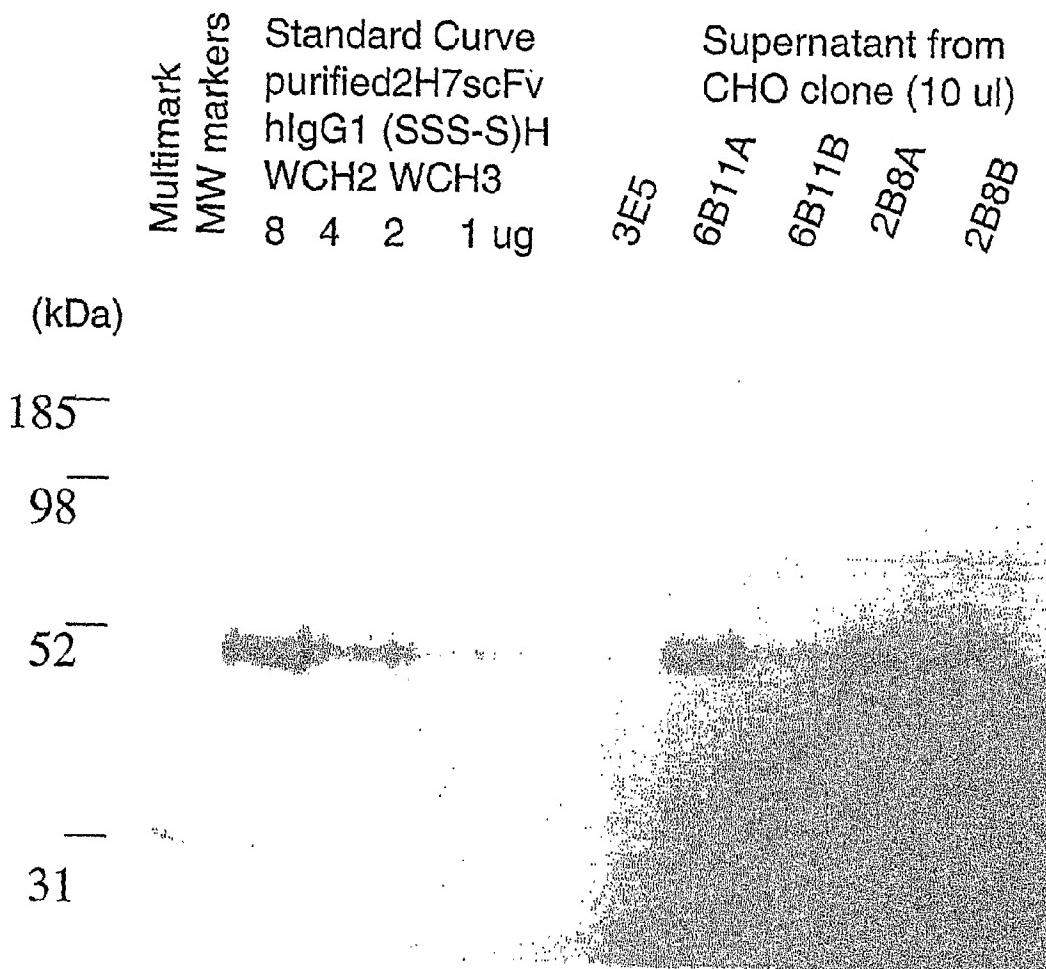
|                           | CHO clone name |      |      |       |       |
|---------------------------|----------------|------|------|-------|-------|
|                           | 4F2            | 4F5  | 3E5  | 6B11A | 2B8A  |
| Mean LFE                  |                |      |      |       |       |
| 1/100                     | 71.7           | 40.6 | 31.5 | 99.7  | 101.5 |
| 1/500                     | 27.1           | 12.4 | 11.2 | 40.8  | 43    |
| approx.<br>conc.<br>μg/ml | 600            | 225  | 125  | 1000  | 1250  |

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Fig. 51

Production Levels of 2H7scFv VH L11S hIgG1  
(SSS-S)H WCH2 WCH3  
From CHO Clone Culture Supernatants

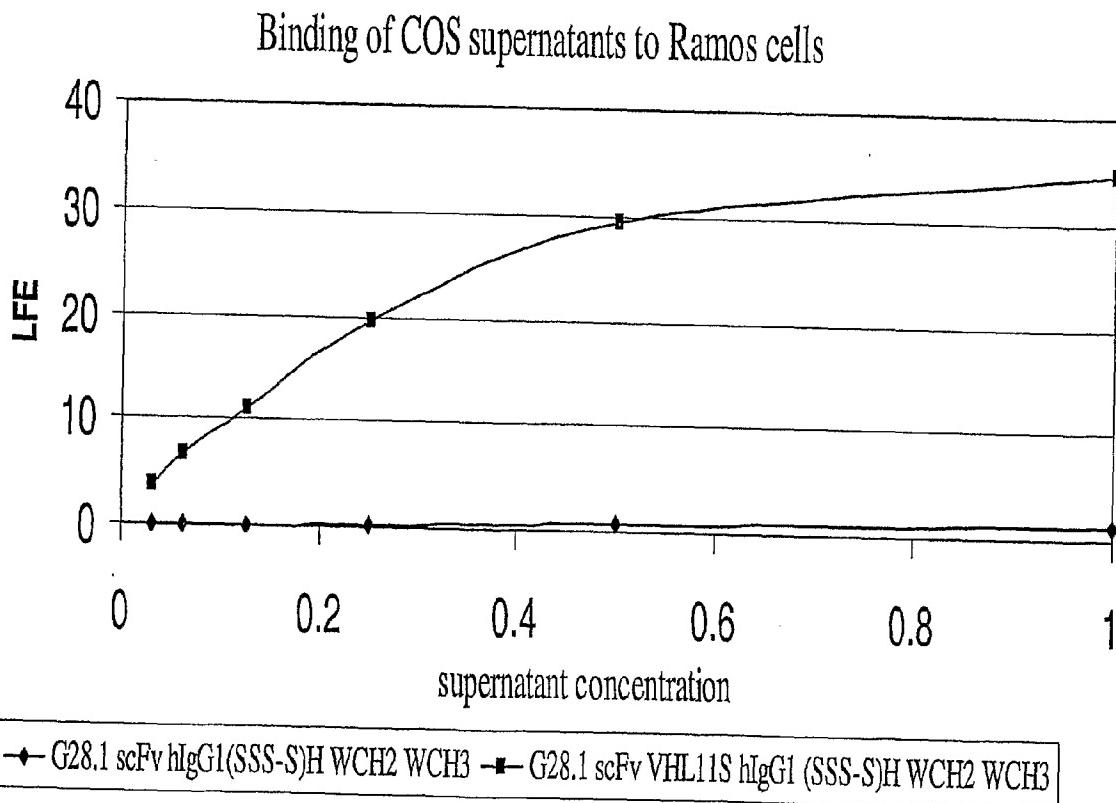


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Fig. 52

Effect of VHL11S Mutation on G28-1 scFvIg Construct Protein Production from COS cells



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Fig. 53

### Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants  
transfected with G28-1scFv hlgG1 (SSS-S)H WCH2 WCH3  
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Fig. 53A.

Purified G28-1 (11/6/01) scFv IgG1 (SSS-S)H WCH2 WCH3  
80ng 40ng 20ng 10ng A B C D E

G28-1 scFv hlgG1 (SSS-S)H WCH2 WCH3 1 ul/well

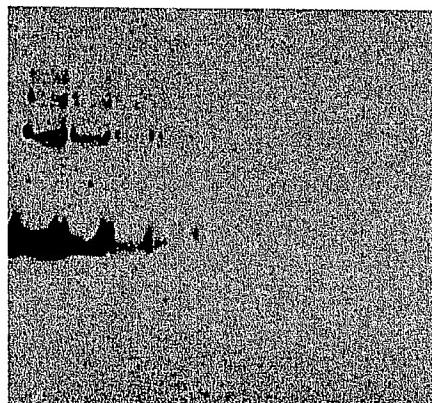
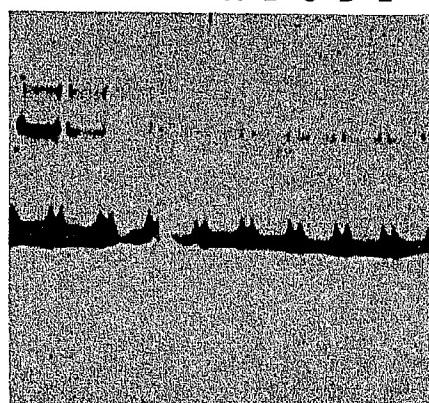


Fig. 53B.

Purified G28-1 (11/6/01) scFv hlgG1 (SSS-S)H WCH2 WCH3  
80ng 40ng 20ng 10ng A B C D E

G28-1VHL11S scFv hlgG1 (SSS-S)H WCH2 WCH3 1 ul/well

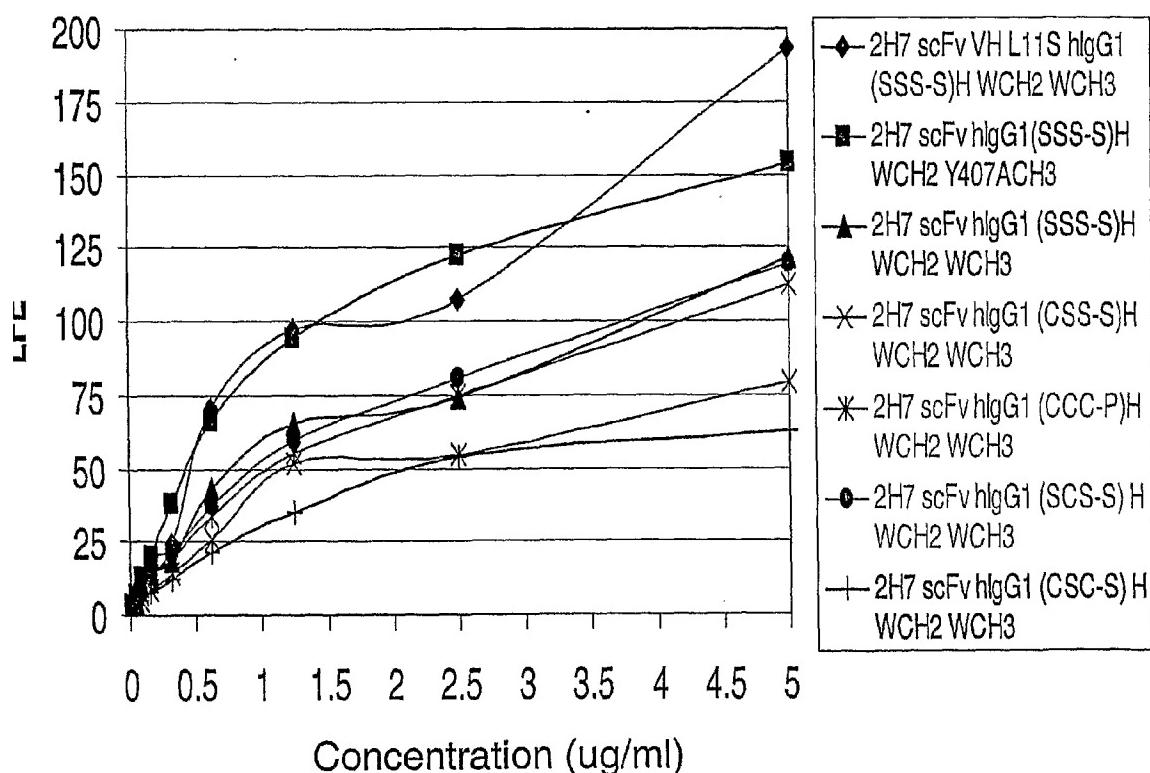


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Fig. 54

Binding of 2H7 scFvIg Constructs with Altered Hinges and CH3 domains to CD20 CHO Cells

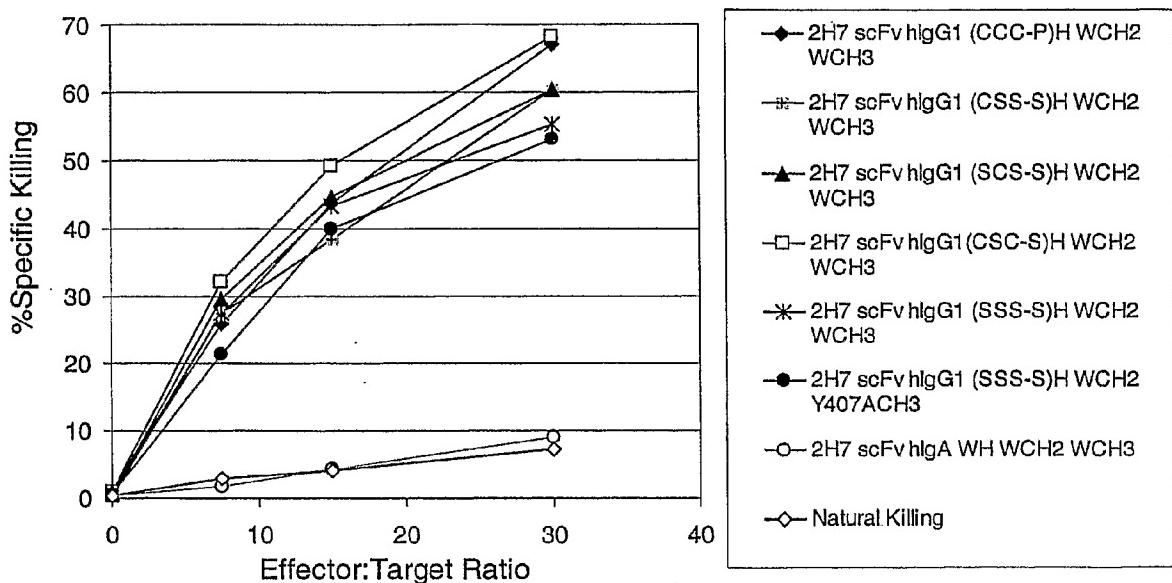


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Fig. 55

ADCC Activity of 2H7 scFvlg constructs Against  
BJAB Targets and PBMC Effectors



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Fig. 56

Complement Activity of 2H7 scFvIg Constructs  
With Ramos Target Cells

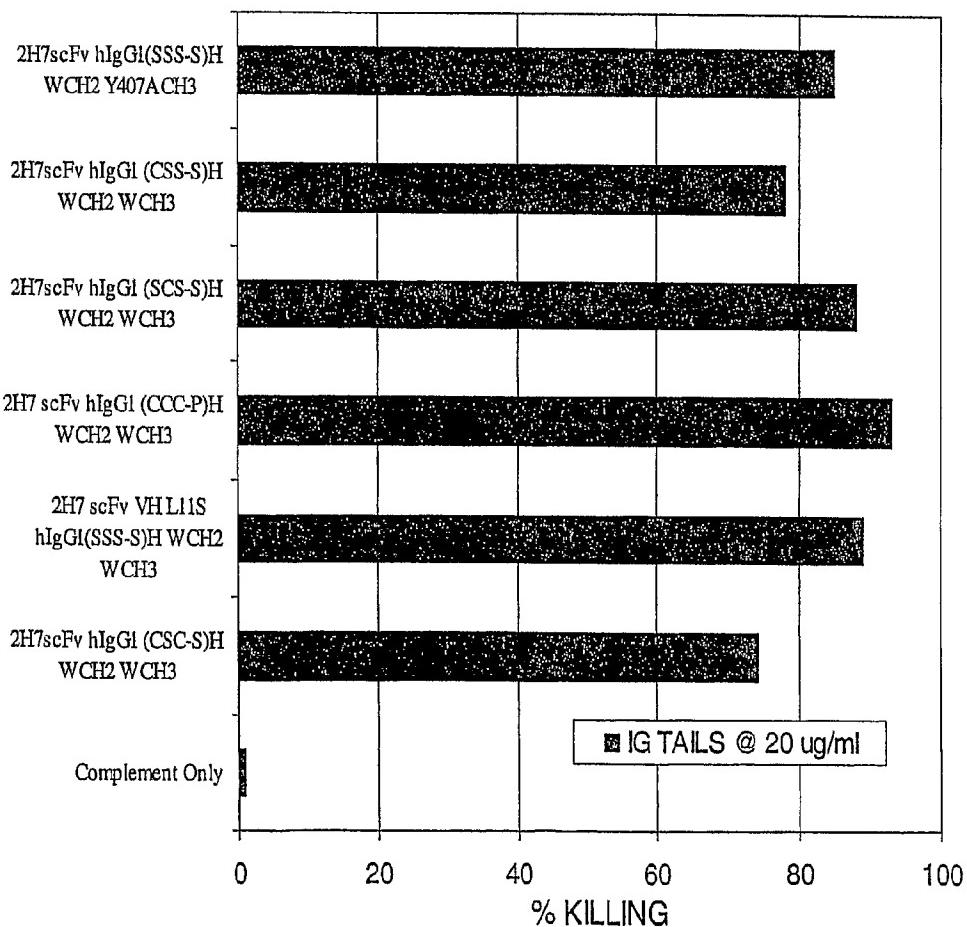
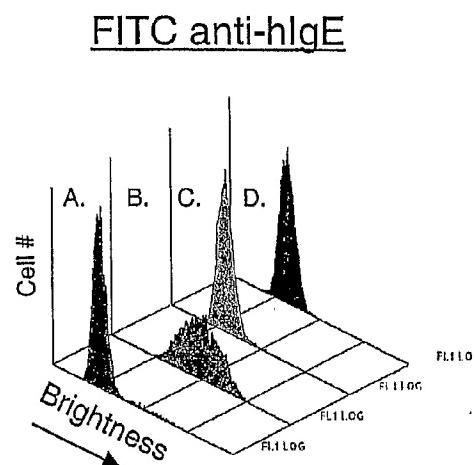
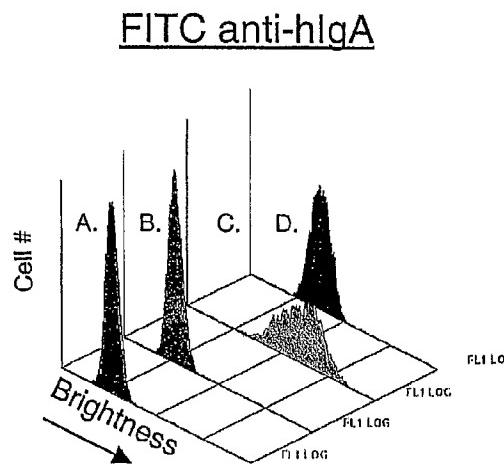
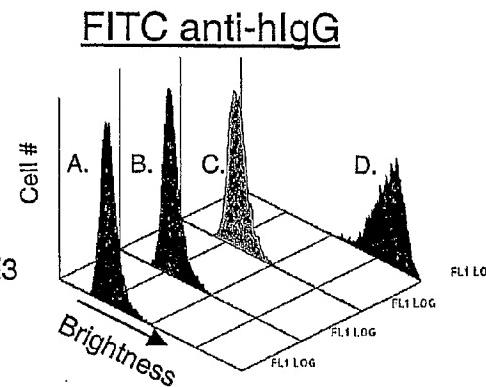


Fig. 57

Binding of 2H7 scFvIg Derivatives to CD20CHO Cells

- A. ■ No fusion protein
- B. □ 2H7 scFv hlgE CH<sub>2</sub>CH<sub>3</sub>CH<sub>4</sub>
- C. ▨ 2H7 scFv hlgA WH WCH<sub>2</sub> WCH<sub>3</sub>
- D. ▲ 2H7 scFv hlgG1 (SSS-S)H WCH<sub>2</sub> WCH<sub>3</sub>



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Fig. 58

Fig. 58A. 2H7 scFv VH L11S human IgE (WCH2 WCH3 WCH4)  
Binding to CD20 CHO at 30 ug/ml

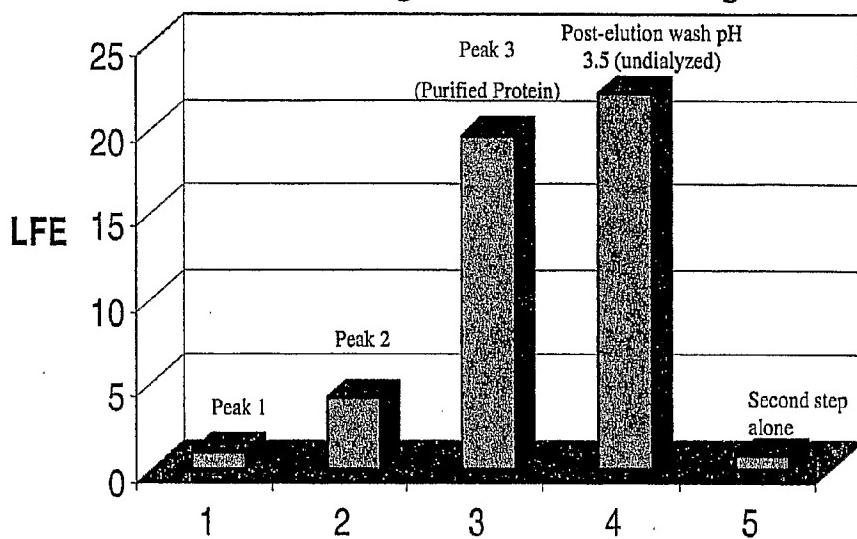
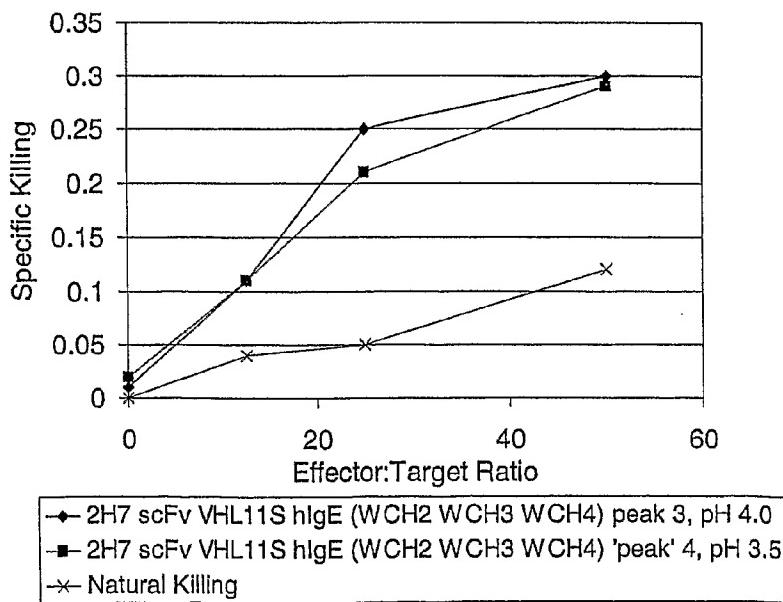


Fig. 58B. ADCC Activity of 2H7 VHL11S IgE (WCH2 WCH3 WCH4)  
Protein Fractions with PBMC Effectors and Bjab Targets

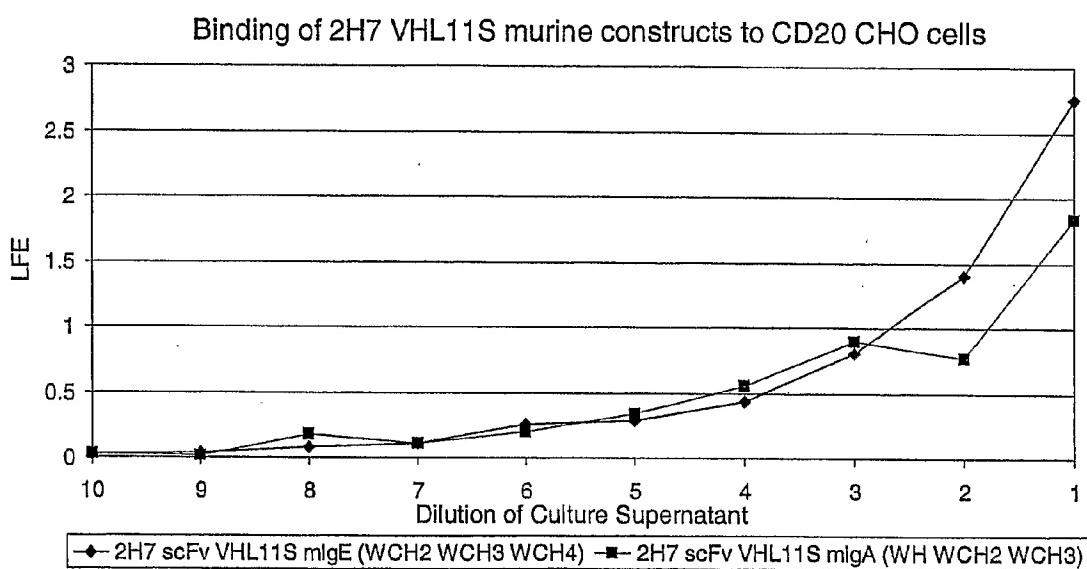


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Fig. 59

Binding Data for COS derived  $\alpha$ -CD20 (2H7) scFv VHL11S  
mIg E (WCH2 WCH3 WCH4) and  
mIgA (WH WCH2 WCH3) Tailed Molecules



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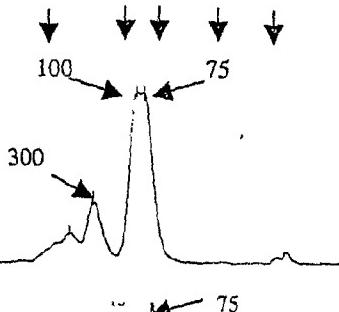
Fig. 60

HPLC Profiles of 2H7 scFvIg Constructs

Size Standards: 670 158 44 17 1.35

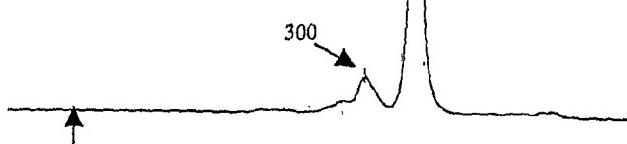
A

2H7 scFv hIgG1  
(SSS-S)H P238SCH2 WCH3



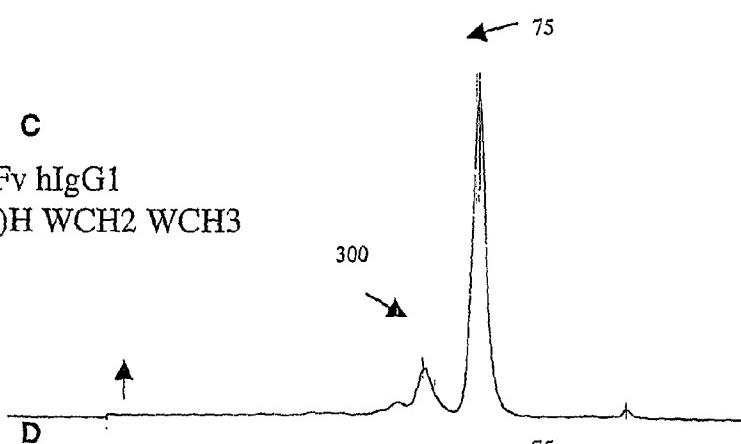
B

2H7 scFv hIgG1  
(CSS-S)H WCH2 WCH3



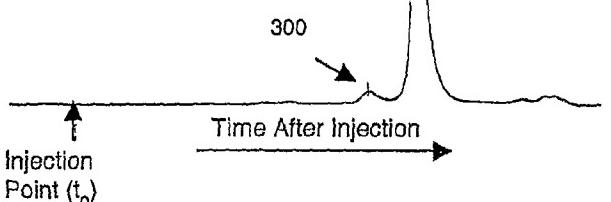
C

2H7 scFv hIgG1  
(SCS-S)H WCH2 WCH3



D

2H7 scFv hIgG1  
(SSS-S)H WCH2 (Y407A)CH3

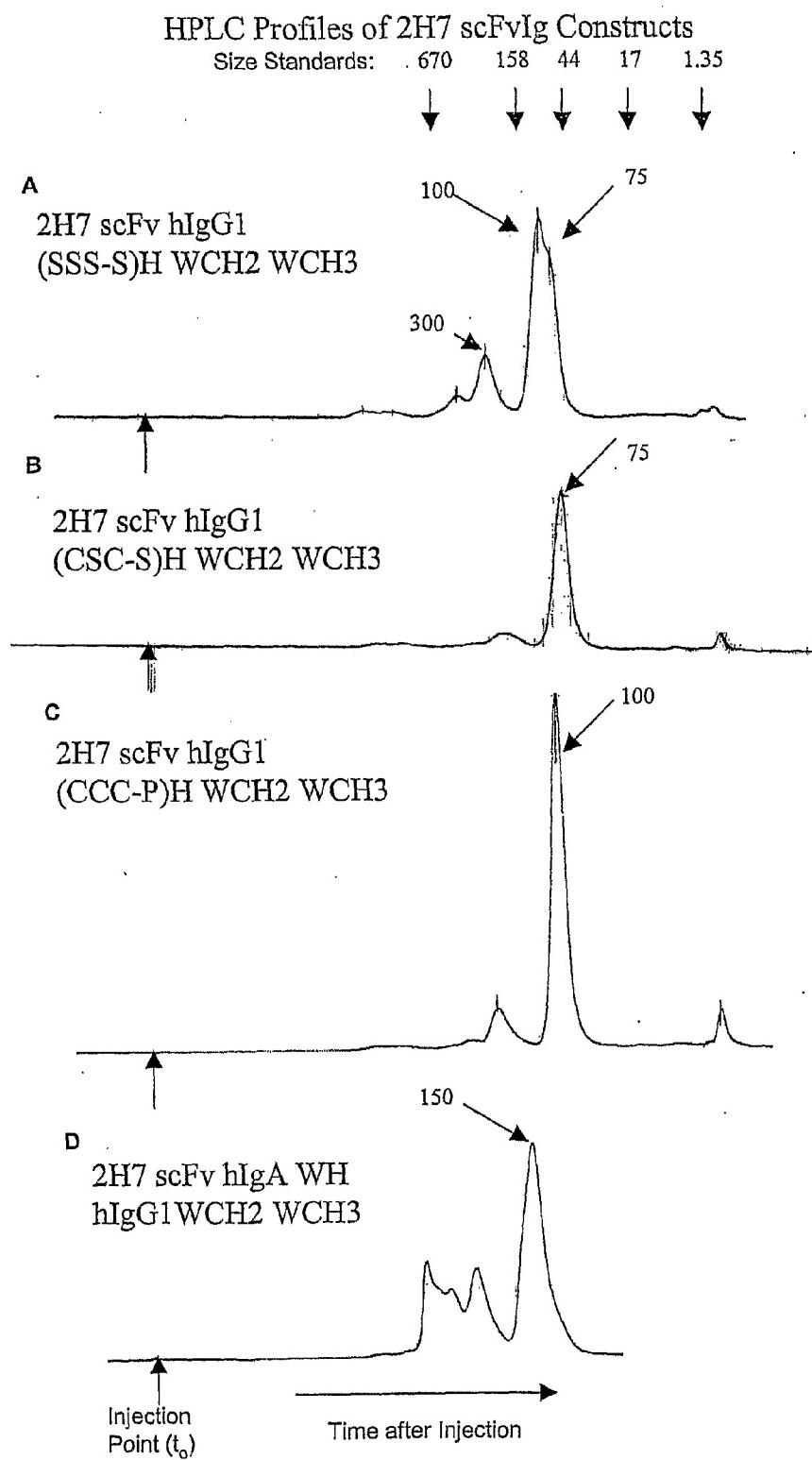


Injection Point ( $t_0$ )

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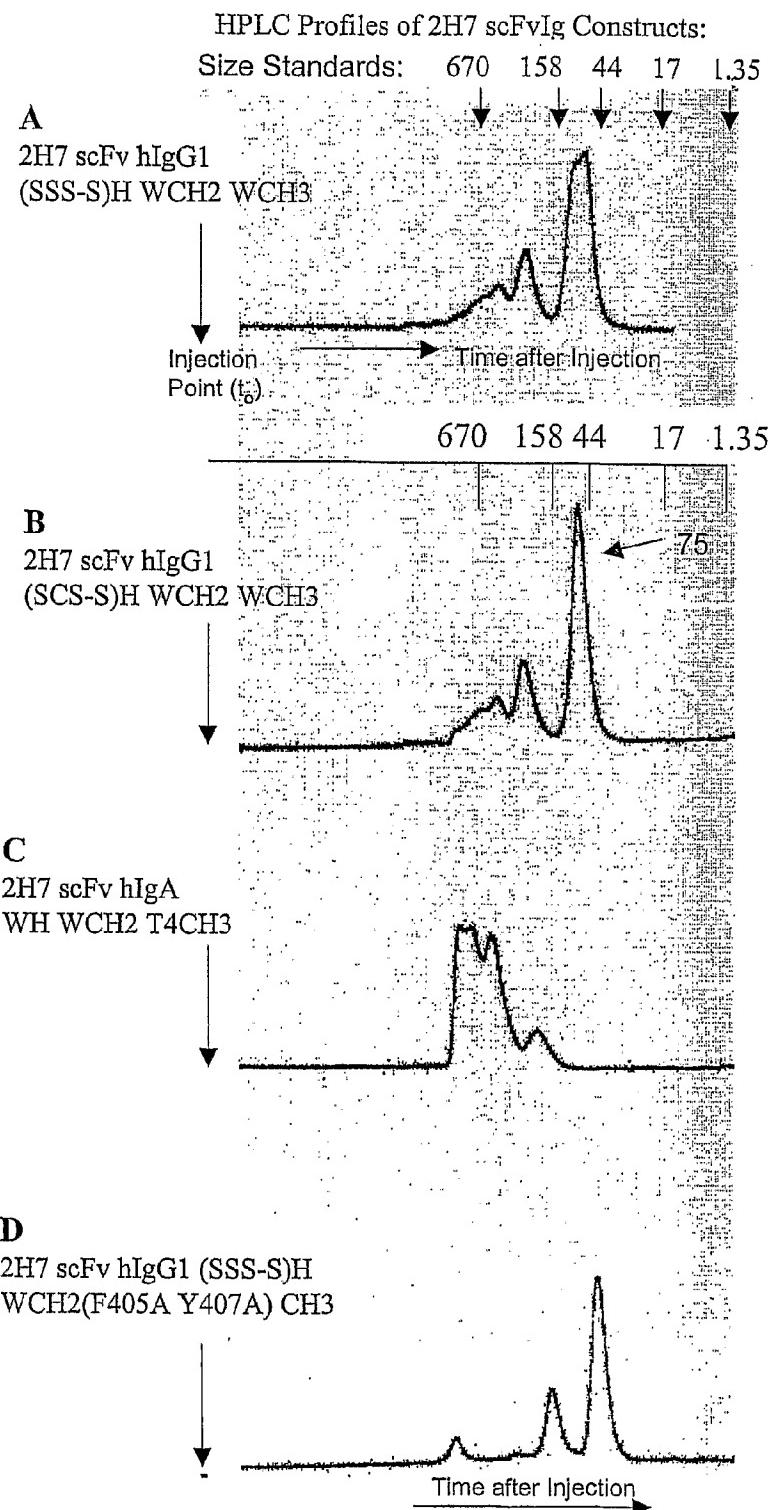
Fig. 61



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Fig. 62

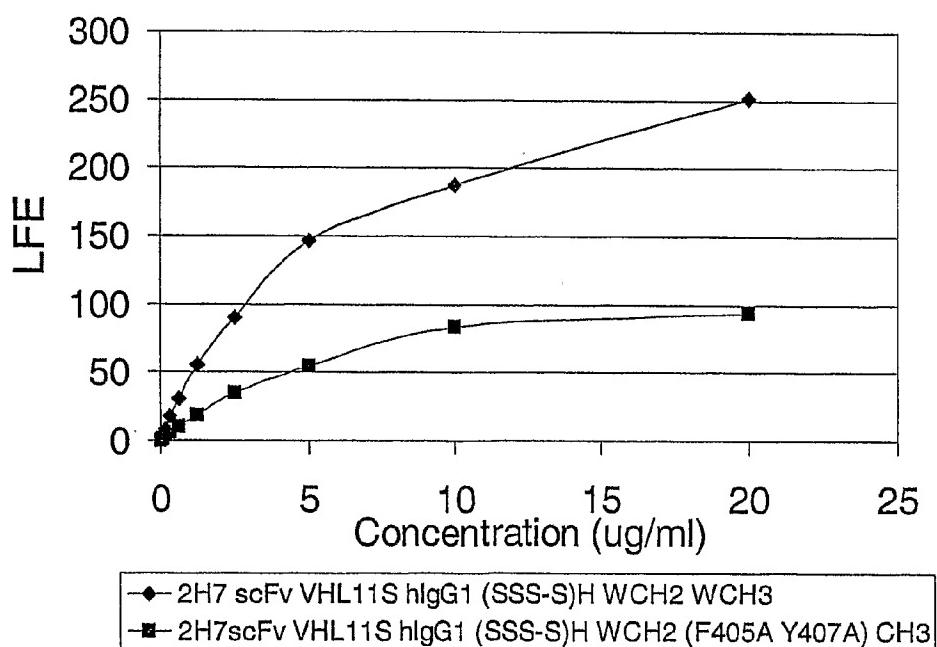


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Fig. 63

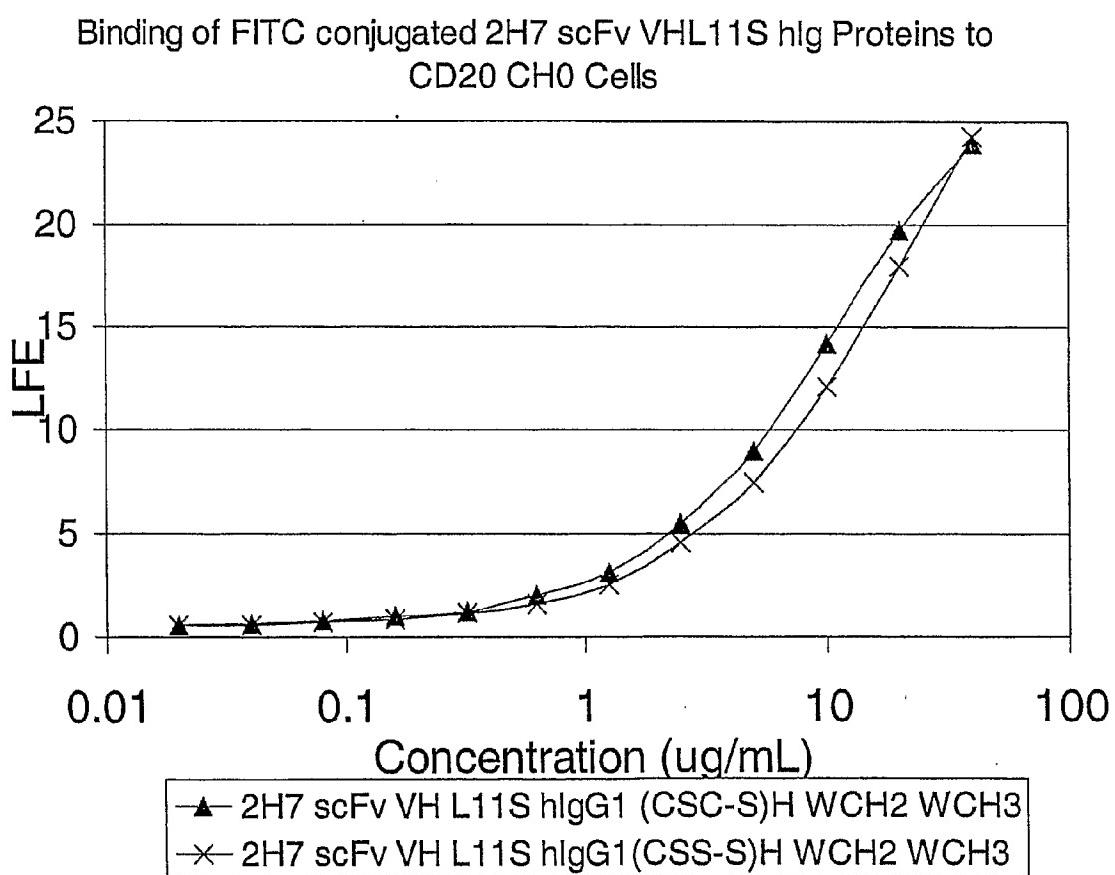
Binding of Purified Proteins from COS Supernatants  
to CD20 CHO cells:  
Differential Effects of CH3 Mutations on Binding



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Fig. 64



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Fig. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots of 2H7 scFv VHL11S hIg Constructs (10 ug/lane)

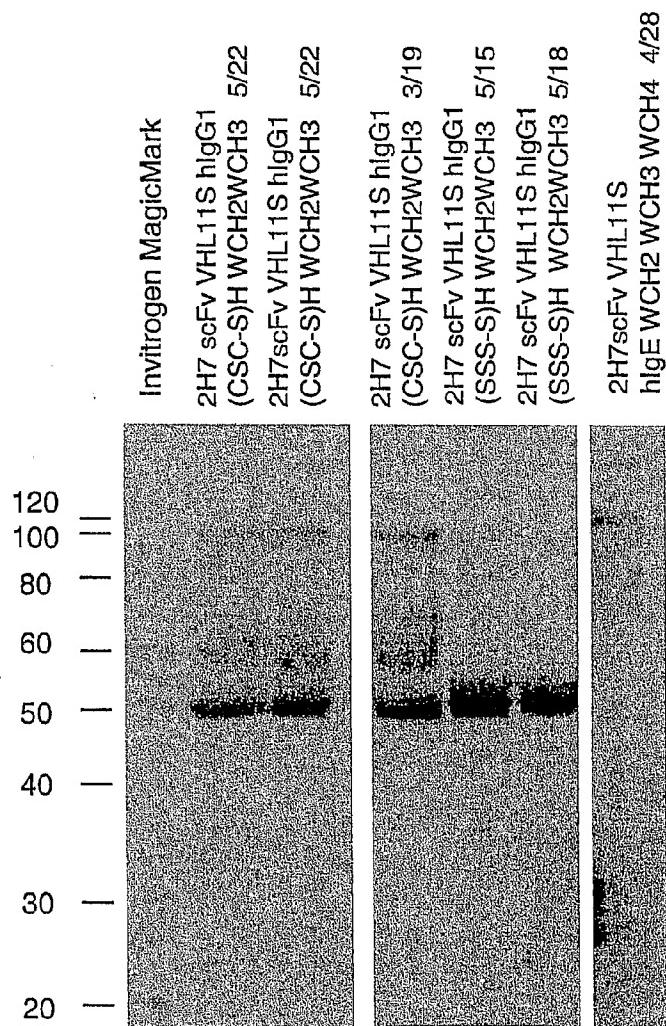
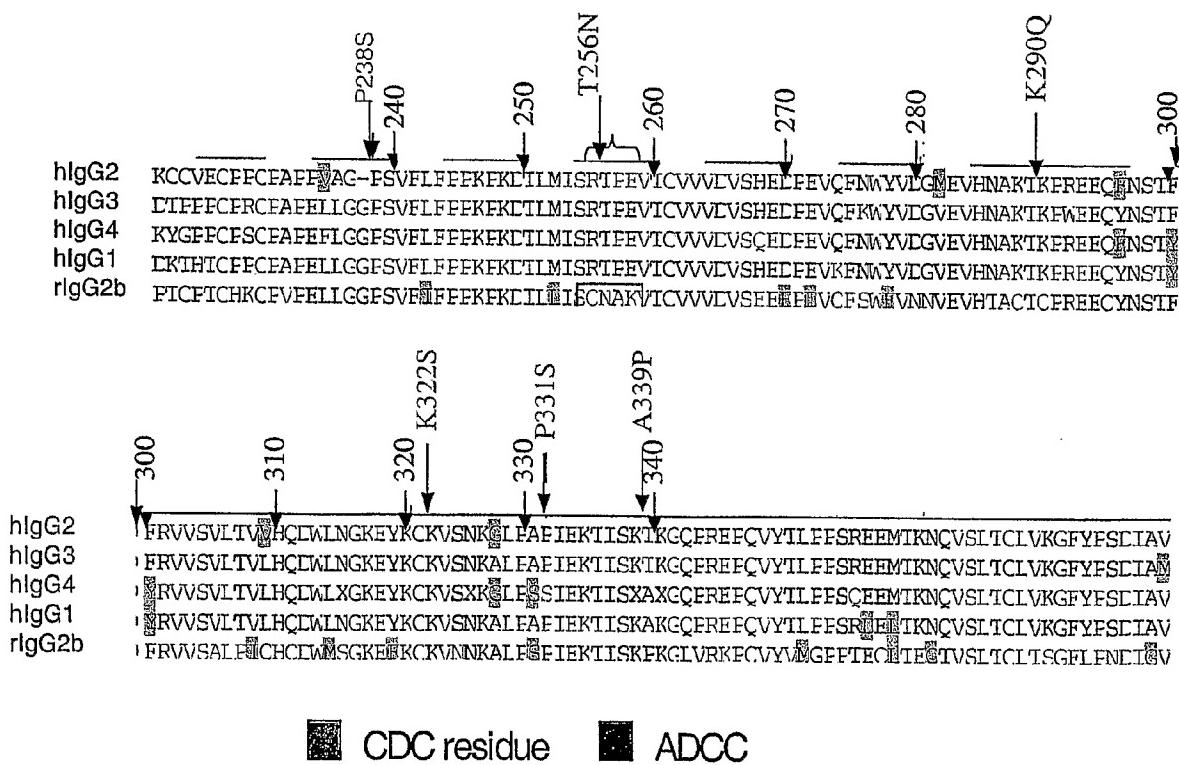


Fig. 66

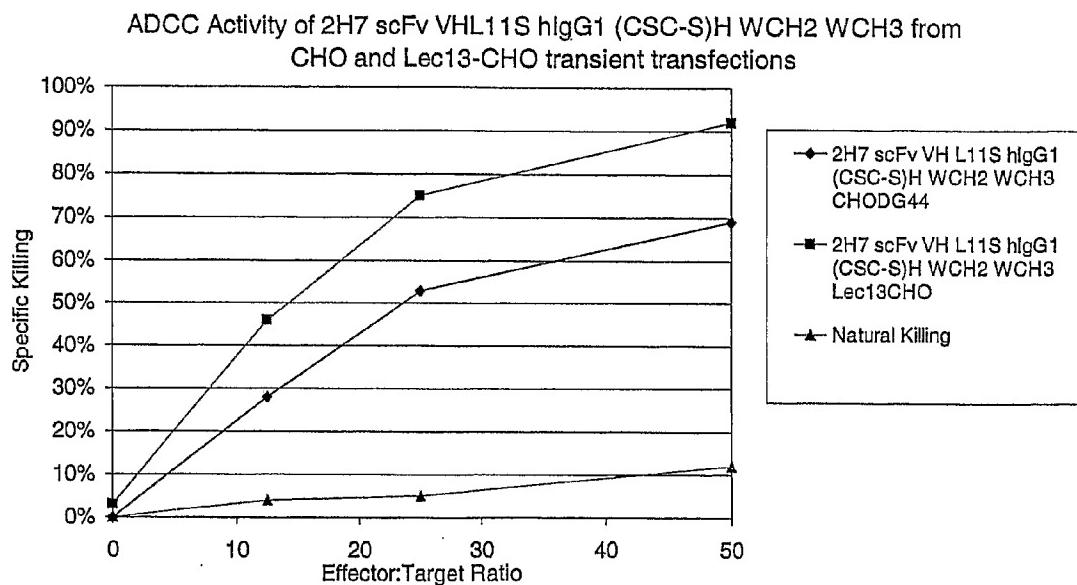
Alterations in Human IgG Fc sequence  
that differentially change effector function efficiency



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Figure 67.

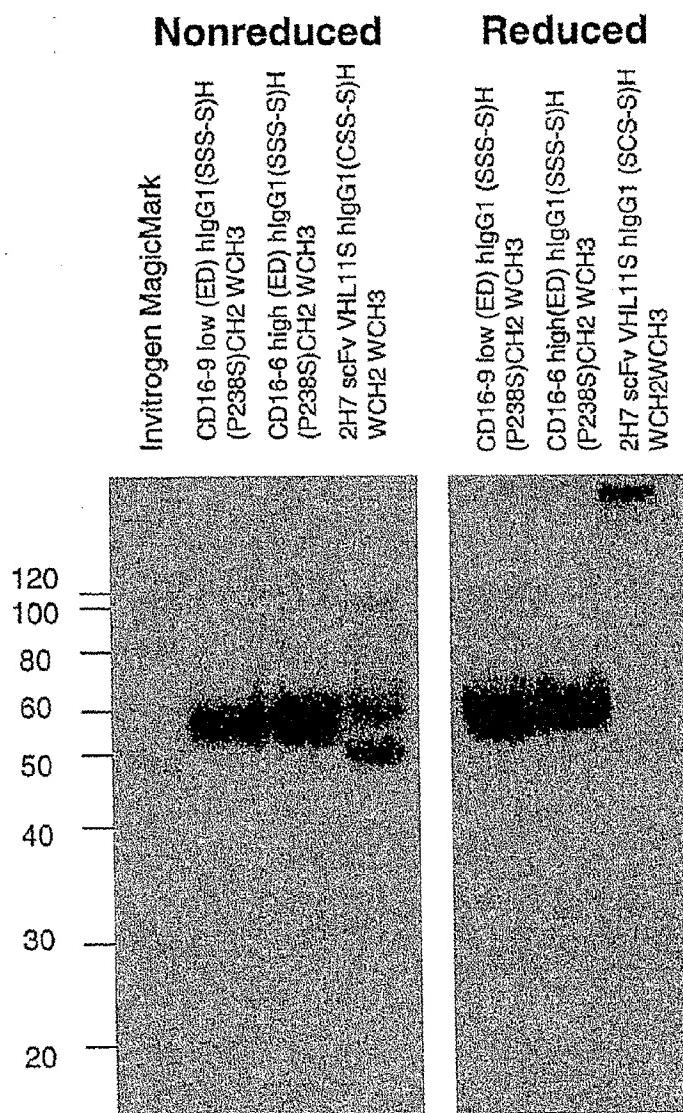


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Fig. 68

CD16(ED) hIgG1(SSS-S)H P238S CH2 WCH3 high and low affinity alleles expressed as soluble molecules



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Fig. 69

Binding of soluble CD16-FITC high and low affinity fusion proteins to 2H7 scFv VHL11S IgG1 (CSC-S)H WCH2WCH3 or (SSS-S)H (P238S)CH2WCH3 on CD20CHO Targets

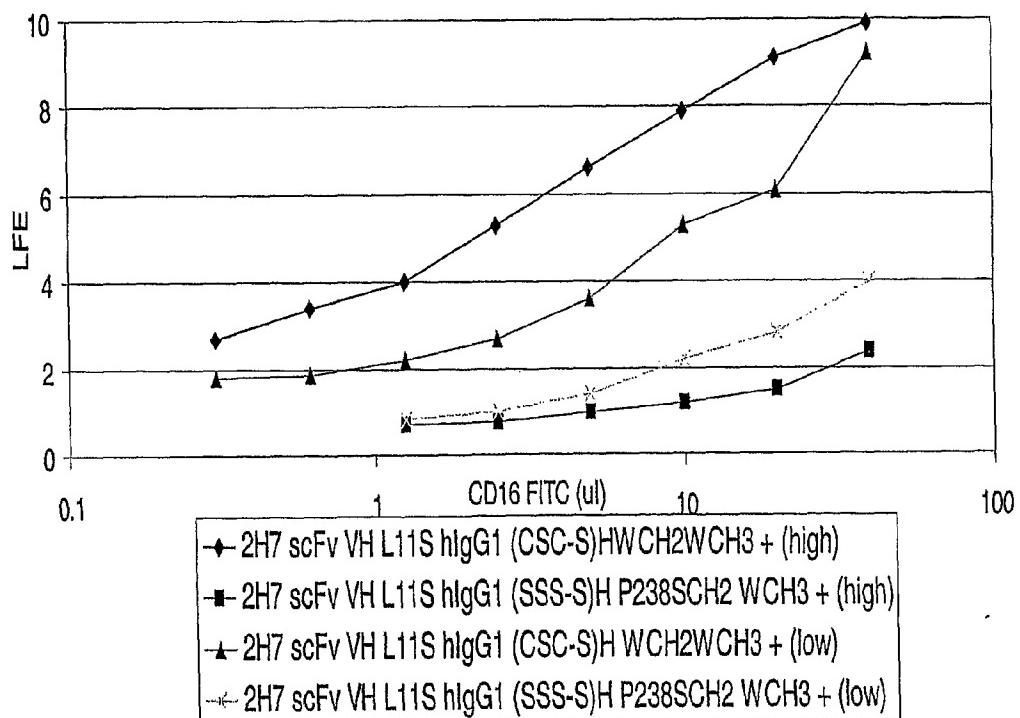
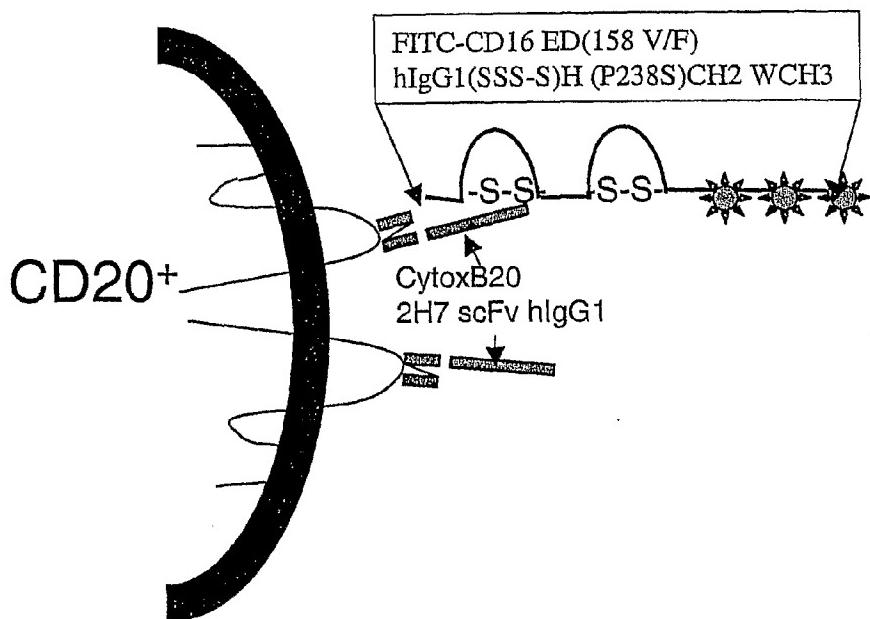
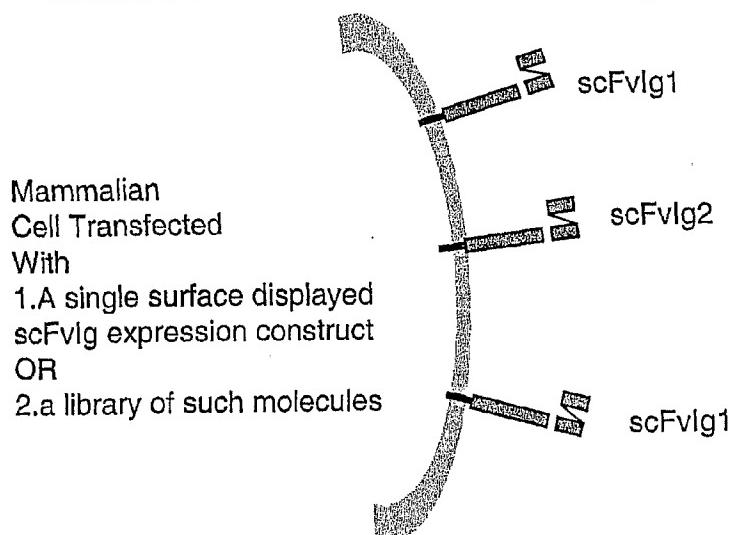


Fig. 70

Binding of FITC Labeled, Recombinant Human CD16(ED) extracellular domain -Ig Fusion Protein to CytoxB Derivatives on CD20 CHO Cells



Expression of surface displayed SMIPs links modified cDNAs with the altered fusion proteins



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Fig. 71

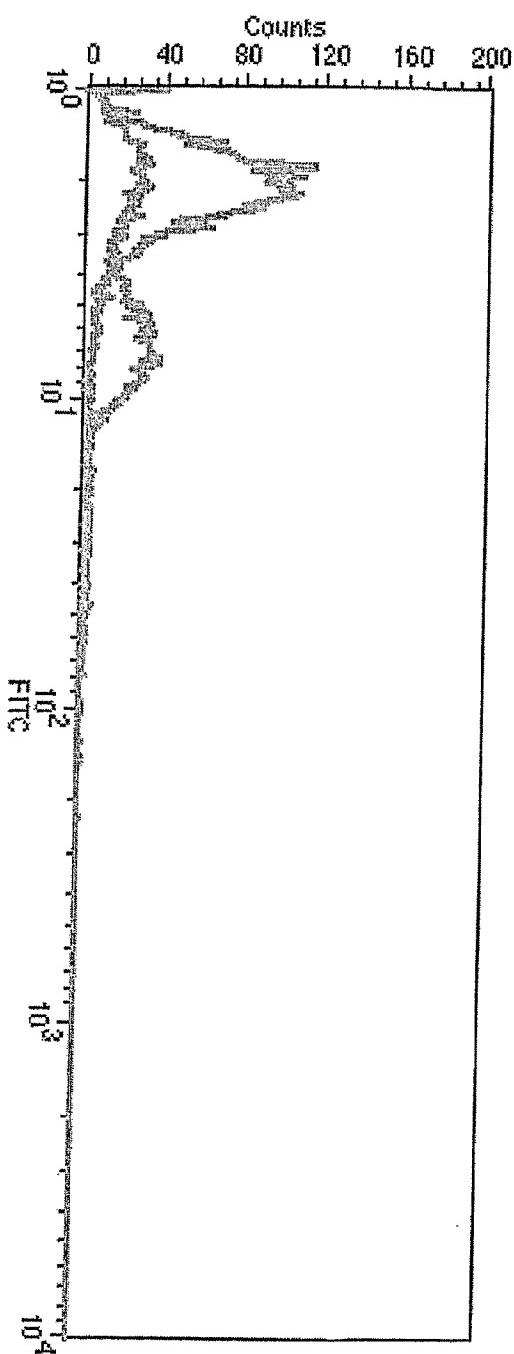
## CD37 mAbs and scFvIg Induce Apoptosis

|        | Bjab Staining   | Annexin V Positive |                   |
|--------|-----------------|--------------------|-------------------|
| scFvIg | No scFvIg       | 17.5               |                   |
|        | 2H7 MH          | 27                 |                   |
|        | G28-1 MH        | 30.6               |                   |
|        | G28-1 IgAH      | 28.9               |                   |
|        | HD37 MH         | 29.1               |                   |
|        | (2H7+G28-1)MH   | 41                 |                   |
|        | (2H7+HD37) MH   | 37.1               |                   |
|        | (G28-1+HD37) MH | 35.3               |                   |
|        |                 |                    |                   |
|        |                 |                    |                   |
| mAbs   | Ramos           | plus GAM           |                   |
|        | cells alone     | AnnexinV Positive  | AnnexinV positive |
|        |                 | 3                  | 3.3               |
|        | 2H7 Mab         | 1.4                | 3.1               |
|        | G28-1 Mab       | 18.3               | 8.7               |
|        | HD37 Mab        | 3.7                | 3.1               |
|        | G28-5           | 3.9                | 8.3               |
|        | 2H7+G28-1       | 32.3               | 35.7              |
|        | 2H7+HD37        | 5                  | 10.5              |
|        | 2H7+G28-5       | 5.7                | 19.4              |
|        | HD37+G28-1      | 26.9               | 50                |
|        | HD37+G28-5      | 8.2                | 18.4              |
|        | G28-1+G28-5     | 39.5               | 68.3              |
|        |                 |                    |                   |

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## Caspase 3 Activity in Ramos Cells after 4 Hour Incubation With CytoxB20G SMIP



Media  
CD20 SMIP

Fig. 72

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## Complement Dependent Cytotoxicity Mediated by CytoxB20G Derivatives

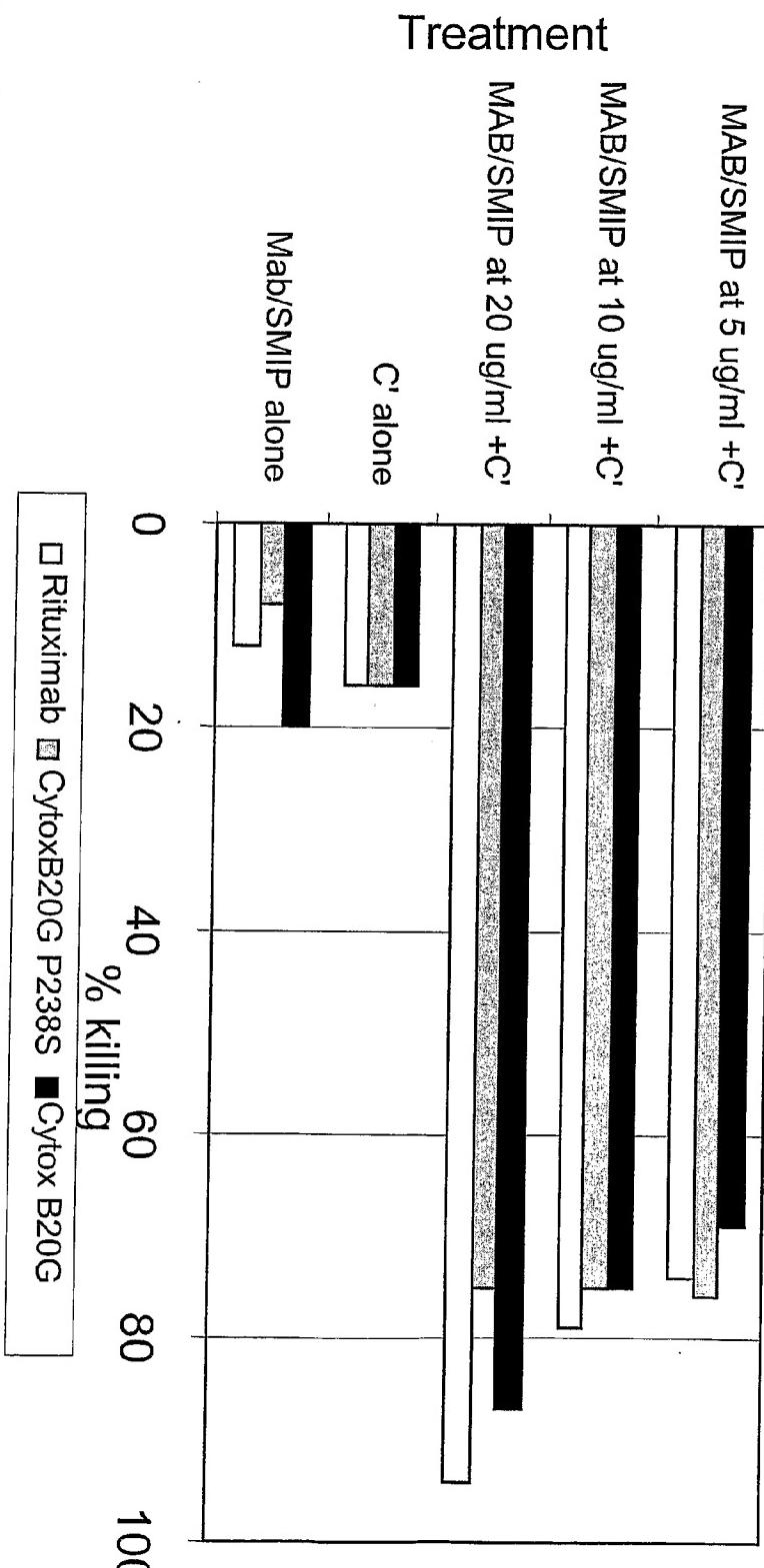


Figure 76: CDC Activity of CytoxB20G SMIPS. CytoxB20G, CytoxB20GP238, or Rituximab were incubated at increasing concentrations with  $10^4$  Bjab Target Cells and a 1:10 dilution of rabbit complement (PefFreez) in a volume of 100 microliters for sixty minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included.

Fig. 73

# ADCC Activity of CytoxB20G SMIPS

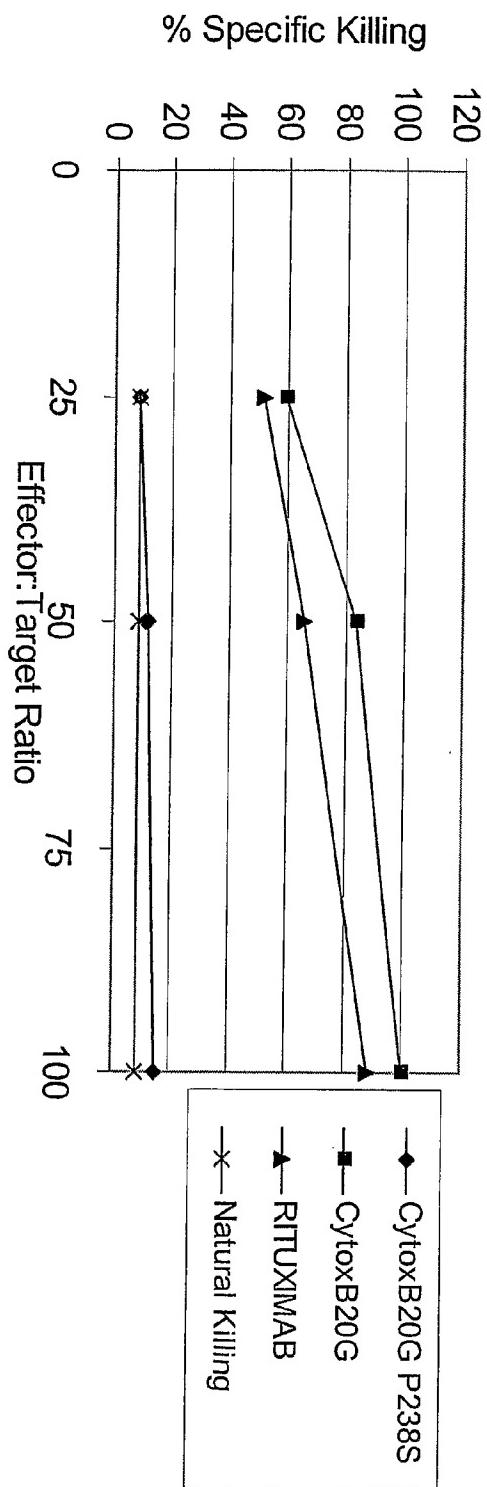


Fig. 74

Figure 77: ADCC Activity of CytoxB20G SMIPS. ADCC activity of CytoxB20G or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 10:1, 50:1, and 25:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with  $^{51}\text{Cr}$  and aliquoted at a cell density of  $5 \times 10^4$  cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10  $\mu\text{g}/\text{ml}$ , and PBMC were added at  $1.25 \times 10^6$  cells/well (25:1),  $2.5 \times 10^6$  cells/well (50:1), or  $5 \times 10^6$  cells/well (100:1), in a final volume of 200  $\mu\text{l}$ . Natural Killing was measured at each effector:target ratio by omission of SMIP or MAb. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 5 hours, and 100  $\mu\text{l}$  culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

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## Binding of soluble FITC-CD16 to CytoxB20G on CD20 CHO Cells

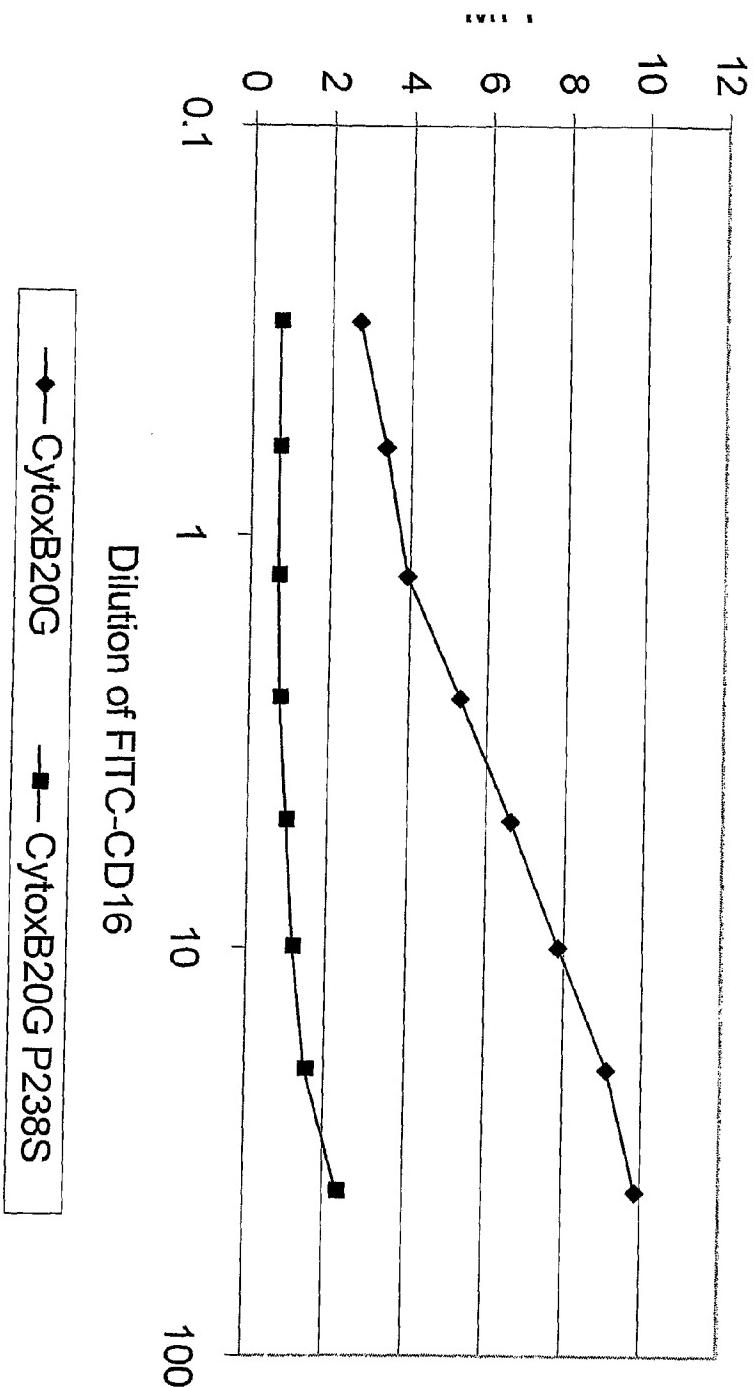


Fig. 75

re 78: Binding of soluble FITC-CD16 to CytoxB20G on CD20 CHO cells. CD20 CHO cells ( $10^6$ ) were incubated ; saturating amounts of CytoxB20G or CytoxB20G P238S(10  $\mu$ g/ml) for one hour on ice in PBS/2% FBS. Cells ; washed in PBS/2% FBS and incubated with serial dilutions of 0.5 mg/ml FITC-CD16 for one hour on ice. Cells ; analyzed using Expo analysis software and normalized fluorescence units graphed as a function of concentration.

## CytoxB20G and CytoxB20G P238S SMIPs bind to U937 Cells Expressing Fc $\gamma$ RI High Affinity FcR

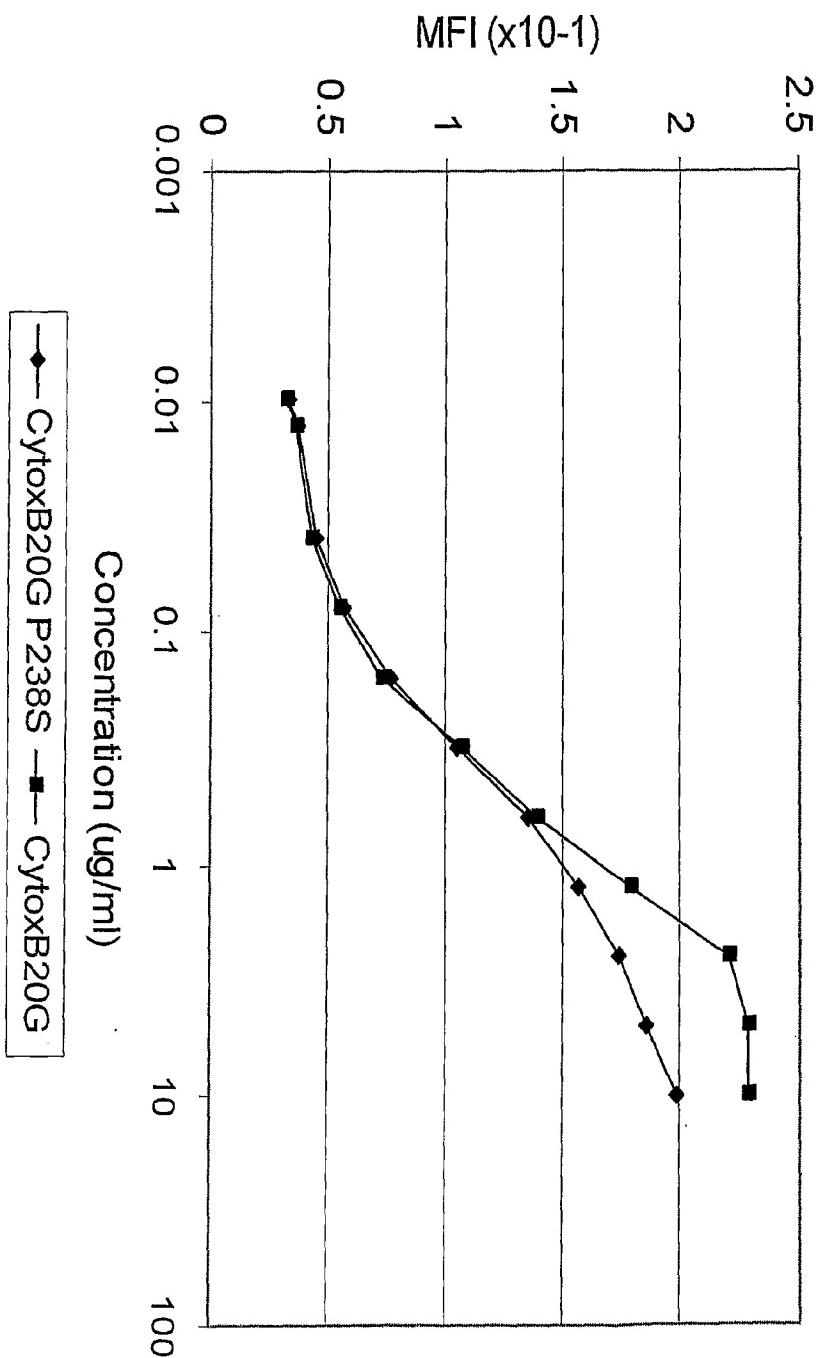
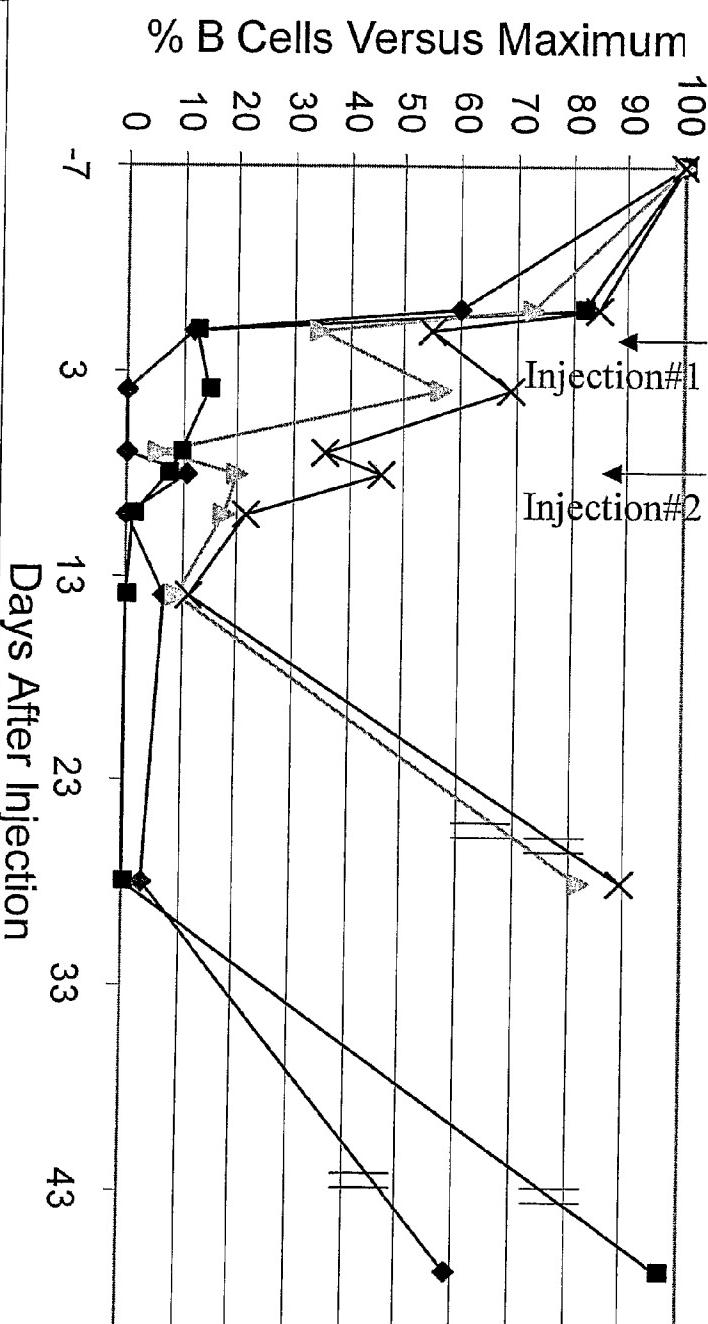


Fig. 76

re 79: CytoxB20G SMIPs bind similarly to U937 cells expressing the high affinity FcR (Fc $\gamma$ RI, CD64). U937 cells expressing CD64 were incubated in PBS/2%FBS for one hour on ice with CytoxB20G or CytoxB20G P238S. Cells washed and incubated for one hour on ice with FITC-goat anti-human IgG1 (Fc specific) (Caltag) at a final dilution 100. Cells were washed and fluorescence analysed on a Beckman-Coulter EpicsC flow cytometer. Data was analyzed using Expo analysis software, and fluorescence intensity graphed as a function of SMIP concentration.

## B Cell Depletion Mediated by CytoxB20G SMI<sup>Ps</sup>



;0: CytoxB20G or CytoxB20G P238S were administered to macaques by intravenous injection at 6 mg/kg, with injections given one week apart. The effect on circulating B cells was measured by detection of CD40 positive B cells in peripheral blood. Blood samples were drawn from injected animals at days -7, 0, 1, 3, 7, 8, 10, 14, 28, and 43. B cell count was estimated by performing CBC (complete blood counts) and two color flow cytometry analysis on monkey FITC or PE conjugates of antibodies against CD40, CD19, CD20, IgG, CD3, CD8 were used in various dilutions. Data are plotted as the number of CD40 positive blood B cells tabulated in thousands of cells per ml over time relative to the initial pre-injection time point level of B cells (maximum).

Fig. 77

Figure 81: SEC on CytoxB37G SMIPs containing SSS and SSC hinge Domains from Human IgG1

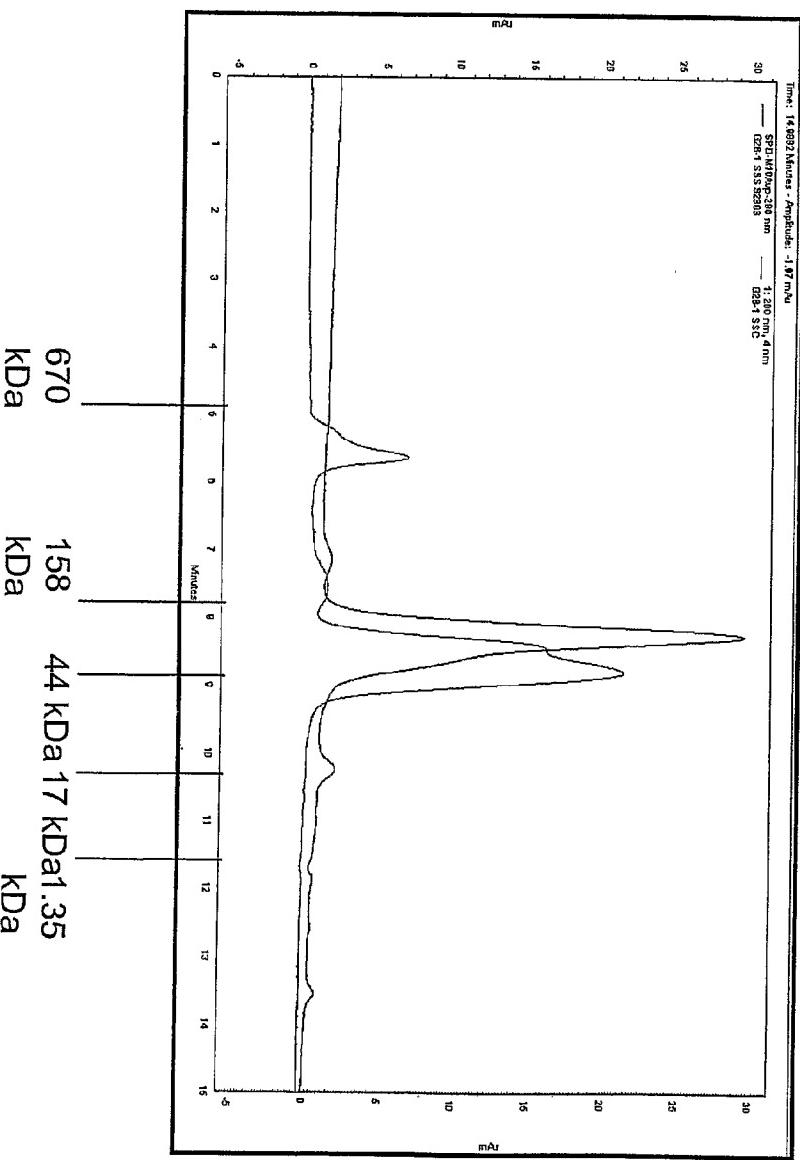
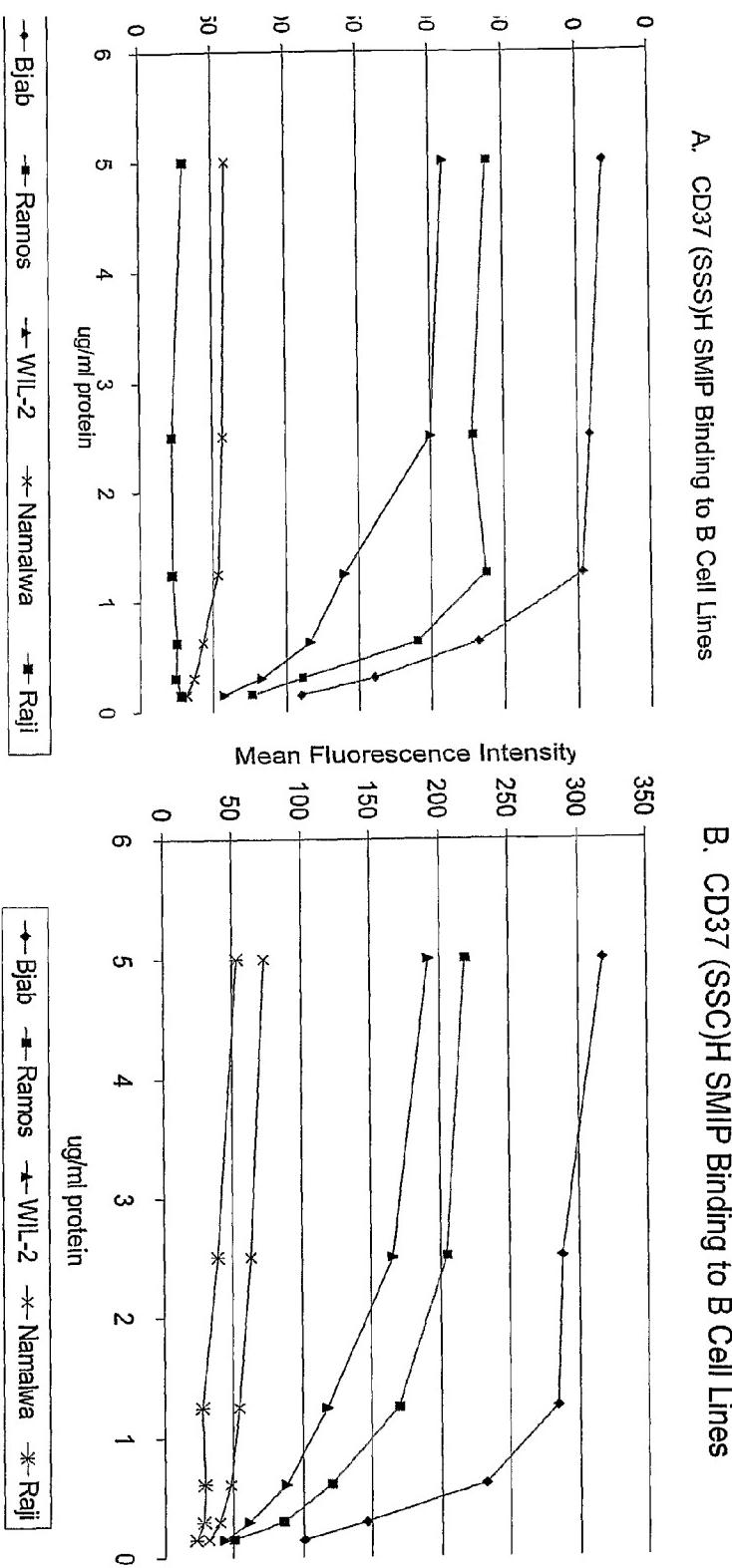


Fig. 78

Figure 81: SEC (Size Exclusion Chromatography) CytoxB37G SMIPs were purified from CHO culture supernatants by Protein A affinity chromatography. Purified aliquots of 10-25 µg were subjected to HPLC over a Tosoh Biosep, Inc. TSK 3000 SWXL HPLC column, pore size 5 µm. The flow rate was 1mL/min, in PBS, pH 7.2 running buffer. Migration rates of molecular weight standards are indicated below the tracing. The CytoxB37G (SSS)H SMIP indicated in blue, while the CytoxB37G (CSS)H is indicated in red.

## figure 82: Binding of CytoxB37G SMIPs to B Cell Lymphoma Cell Lines

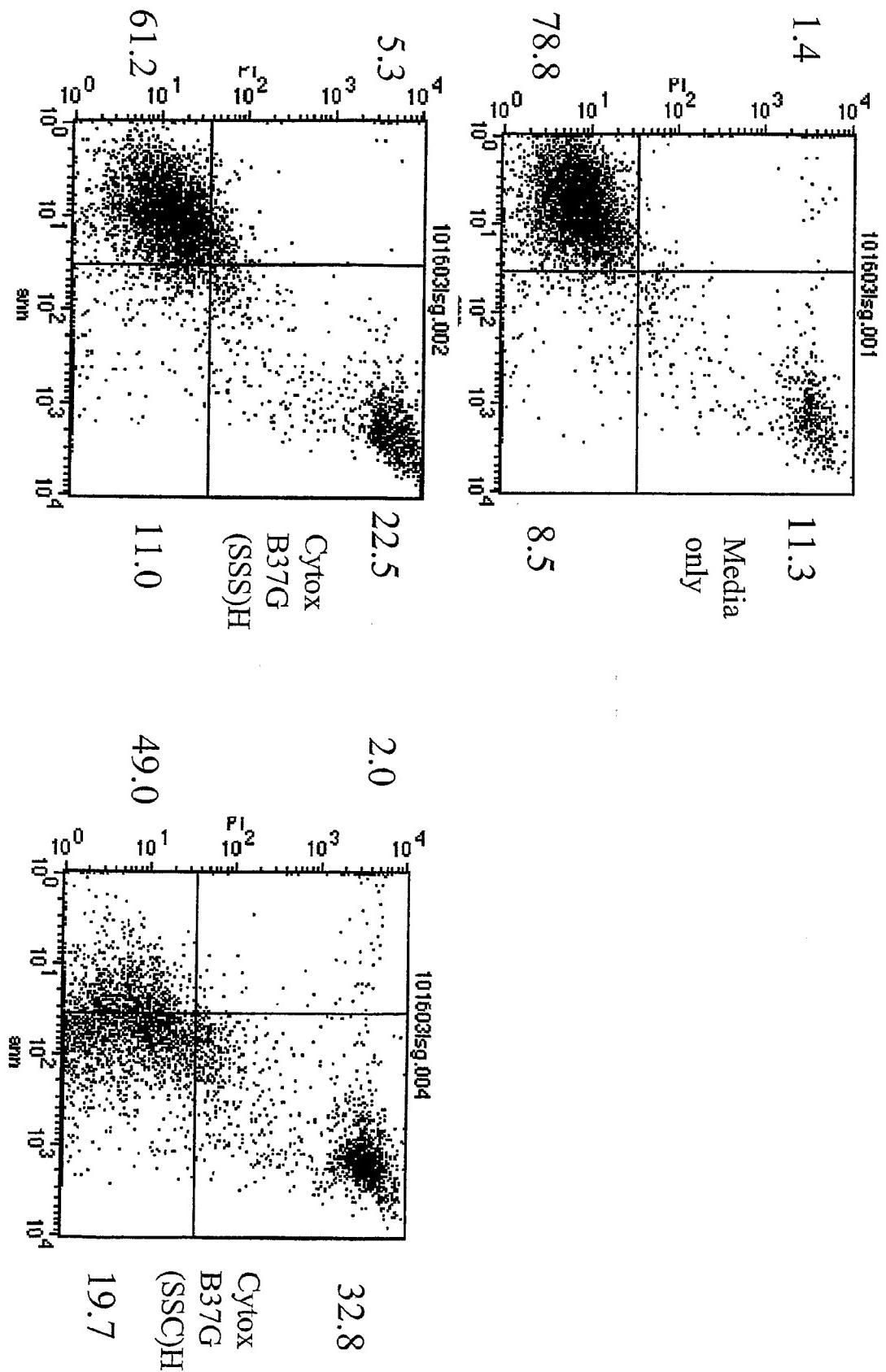


ure 82 : Binding of CytoxB37G SMIPs to B cell lymphoma cell lines. Serial dilutions of CytoxB37 (SSS)H G or CytoxB37 (SSC)H G SMIPs were incubated with  $10^6$  cells of each cell type for 60 minutes on ice in PBS/2%FBS. Samples were washed twice, and incubated with a mixture of FITC goat anti-human IgG F(ab')2 (CalTag) at 1:100 each, on ice for 45 minutes. Samples were washed and analyzed by flow cytometry using a FACsCalibur (Becton-Dickinson)

Fig. 79

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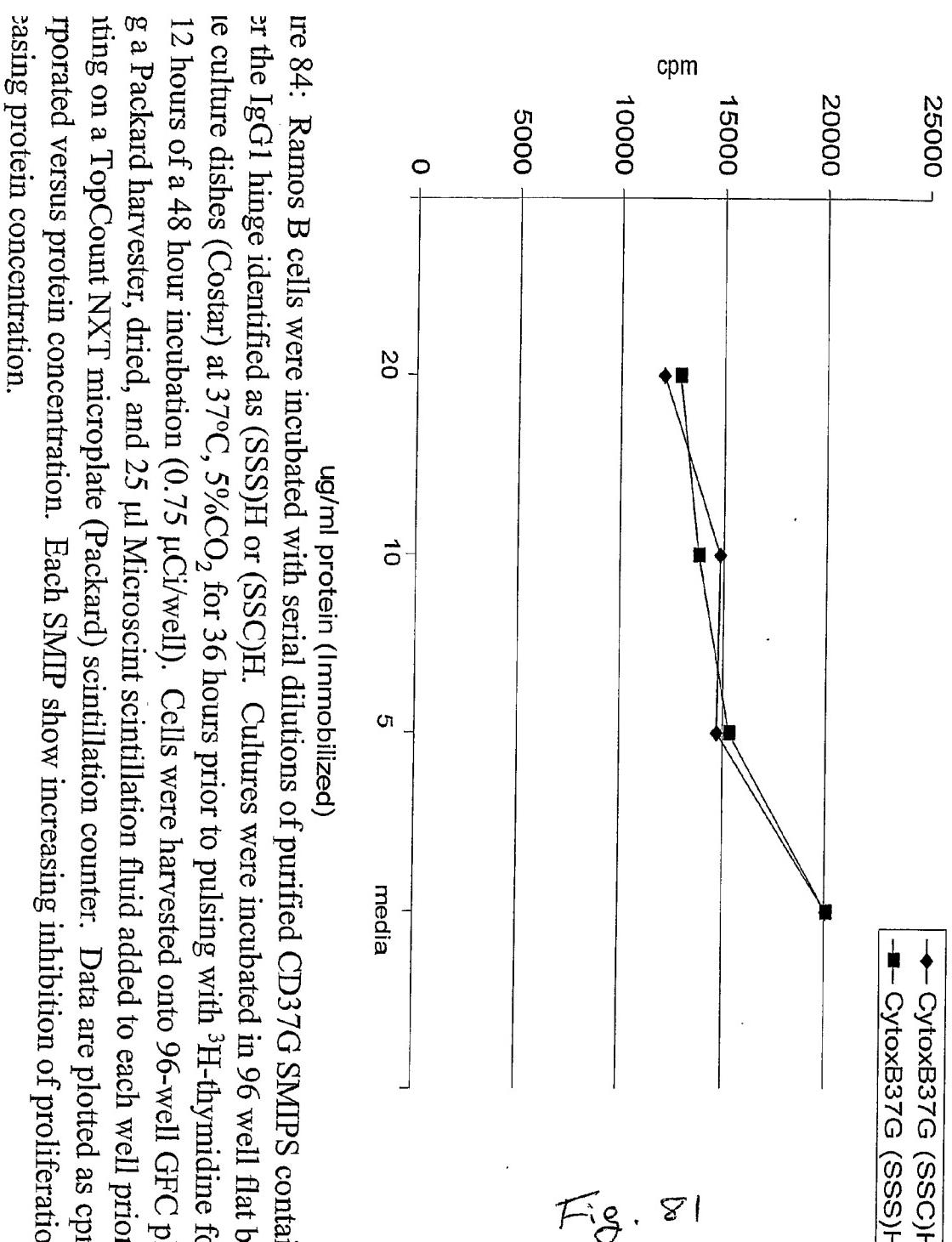
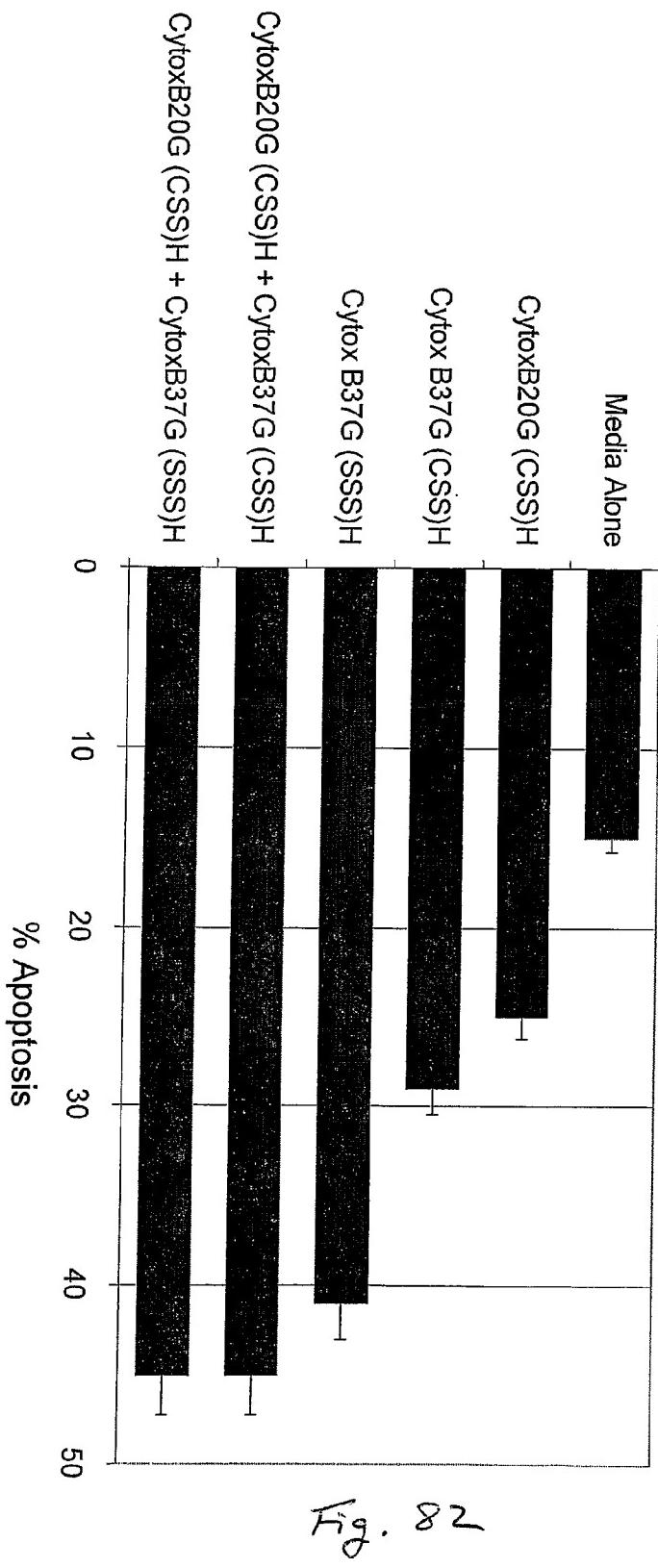


Figure 84: Ramos B cells were incubated with serial dilutions of purified CD37G SMIPS containing the IgG1 hinge identified as (SSS)H or (SSC)H. Cultures were incubated in 96 well flat bottom culture dishes (Costar) at 37°C, 5%CO<sub>2</sub> for 36 hours prior to pulsing with <sup>3</sup>H-thymidine for the 12 hours of a 48 hour incubation (0.75 µCi/well). Cells were harvested onto 96-well GFC plates using a Packard harvester, dried, and 25 µl Microscint scintillation fluid added to each well prior to counting on a TopCount NXT microplate (Packard) scintillation counter. Data are plotted as cpm incorporated versus protein concentration. Each SMIP show increasing inhibition of proliferation with increasing protein concentration.

ure 85: The Induction of Apoptosis in Ramos B-cells after a 20 hour incubation with different combinations of CD20 and CD37 targeted SMIPs



gure 85: Ramos B cells were incubated with CD20 and/or CD37 targeted SMIPs (10  $\mu$ ml) in solution for 20 hours. Cells were then harvested, washed, and incubated in annexin V and propidium iodide using a staining kit from Immunotech prior to two color flow cytometry using a FACsCalibur flow cytometer (Becton-Dickinson). The graph shows the percentage of annexin V positive cells identified by their staining in the right quadrants the dot plots.

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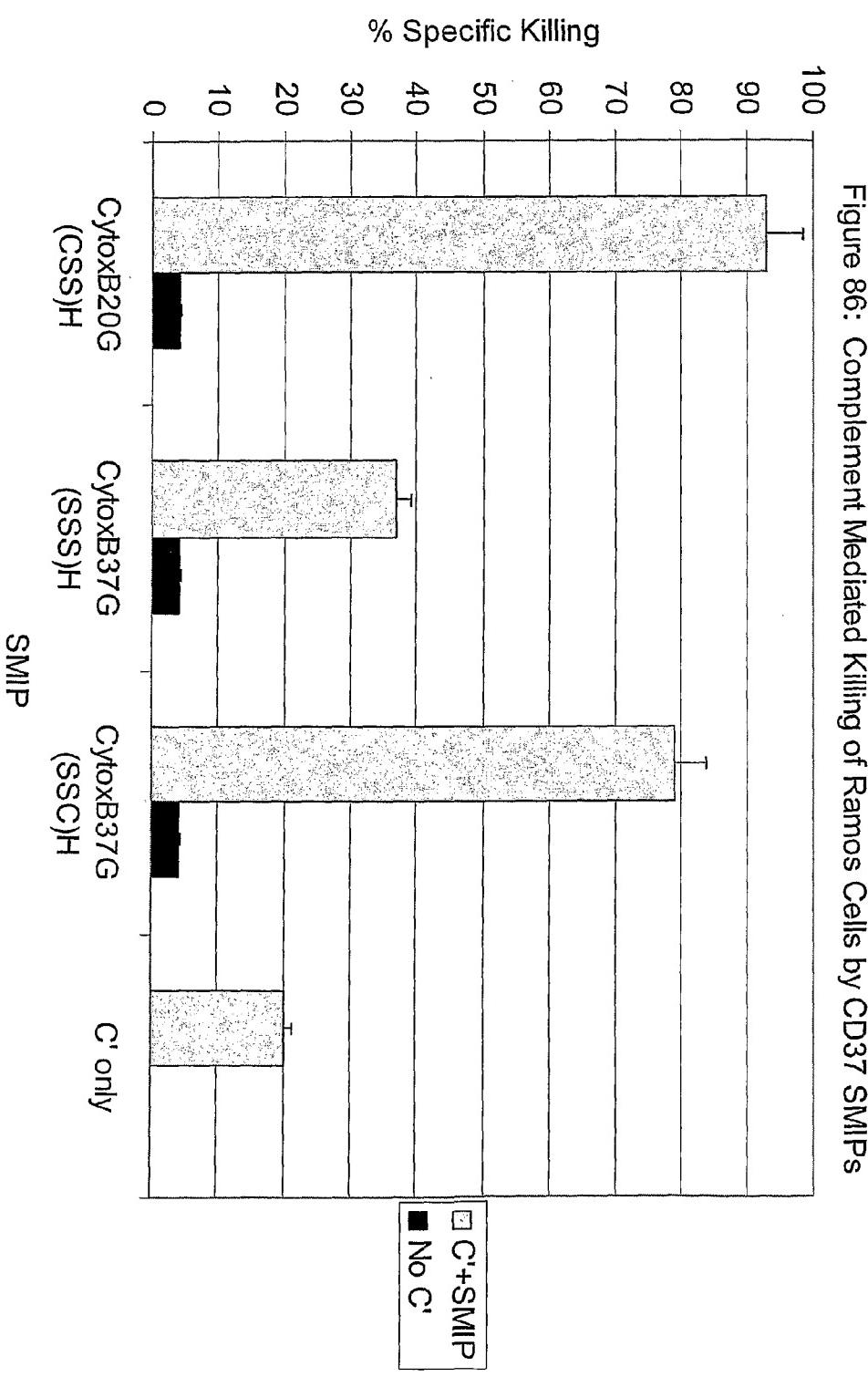


Figure 86: CDC Activity of CytoxB37G SMIPs. CytoxB20G, CytoxB37 (SSS)H G, CytoxB37 (SCSH), CytoxB37 (CSS)H, or CytoxB37 (SSC)H were incubated at 10 µg/ml with 10<sup>4</sup> Ramos Target Cells and a 1:10 dilution of rabbit complement (PelFreez) in a volume of 150 µl for 90 minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included.

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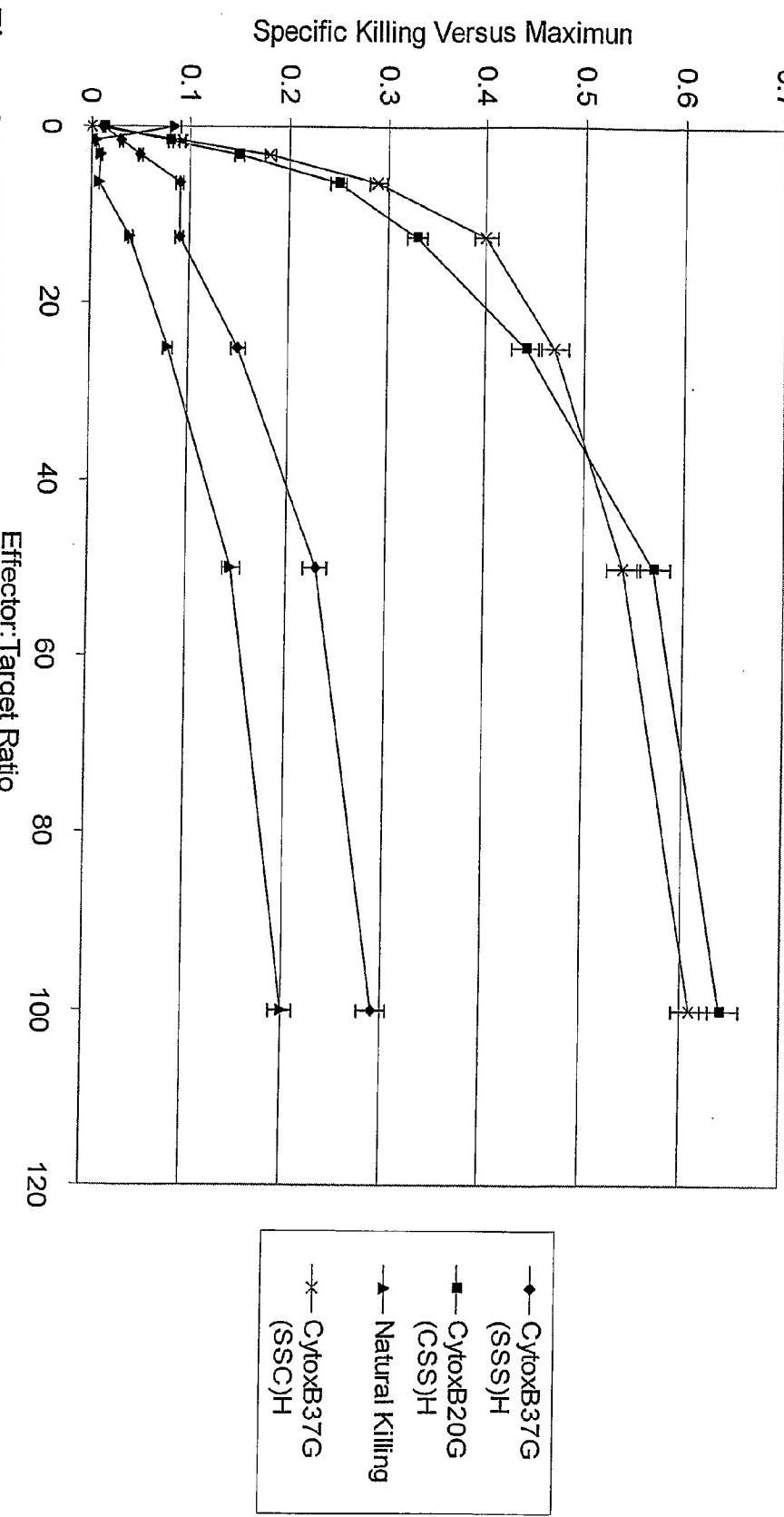
**Figure 87: ADCC Activity of CD37 SMIPs Against Ramos Targets**

Figure 87: CD37 SMIPS at 10 µg/ml were incubated in flat-bottom 96 well plates with  $10^4$   $^{51}\text{Cr}$ -labeled Ramos cells and resting human PBMCs at different effector:target ratios ranging from 0 to 100. All incubations were performed in triplicate at each effector:target ratio. Natural Killing was measured at each effector:target ratio by omission of SMIP. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 6 hours, and 100 µl culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

Fig. 84

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/41600

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/00; A61K 39/395; C07K 16/00

US CL : 530/387.3, 388.85, 391.3; 424/130.1; 536/23.4; 435/320.1, 69.6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.3, 388.85, 391.3; 424/130.1; 536/23.4; 435/320.1, 69.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                       | Relevant to claim No.                                                                                                                              |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Y          | HAYDEN et al. Single-chain mono- and bispecific antibody derivatives with novel biological properties and antitumor activity from COS cell transient expression system. Therapeutic Immunology. 1994, Vol. 94, pages 3-15, especially Figure 1, Methods. | 1-7, 20-28, 31-40, 53-57, 59, 62-63, 65-75, 116-119, 129-137, 140-150, 161-169, 171-181, 238, 240-243, 251-259, 261-267, 282-285, 287-295, 399-411 |
| Y          | US 6,147,203 A (PASTAN et al.) 14 November 2000 (14.11.2000), see entire document, especially abstract, column5-6.                                                                                                                                       | 1-7, 20-28, 31-40, 53-57, 59, 62-63, 65-75, 116-119, 129-137, 140-150, 161-169, 171-181, 238, 240-243, 251-259, 261-267, 282-285, 287-295, 399-411 |



Further documents are listed in the continuation of Box C.



See patent family annex.

|                                                                                                                                                                         |     |                                                                                                                                                                                                                                              |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
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| "E" earlier application or patent published on or after the international filing date                                                                                   | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family                                                                                                                                                                                                    |
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| "P" document published prior to the international filing date but later than the priority date claimed                                                                  |     |                                                                                                                                                                                                                                              |

Date of the actual completion of the international search

29 October 2004 (29.10.2004)

Date of mailing of the international search report

02 NOV 2004

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Authorized officer

Larry R. Helms

Telephone No.

571-272-1600

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## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                 | Relevant to claim No.                    |
|------------|--------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| Y          | US 6,074,644 A (PASTAN et al.) 13 June 2000 (13.06.2000), see entire document, especially column 20.               | 26, 28, 32, 135, 137, 142, 257, 259, 262 |
| Y          | US 5,677,425 A (BODMER et al.) 14 October 1997 (14.10.1997), see entire document, especially abstract, column 3-4. | 65-75, 116, 172-180, 288-295             |
| Y          | US 6,482,919 B2 (LEDBETTER et al.) 19 November 2002 (19.11.2002), see entire document.                             | 180                                      |

INTERNATIONAL SEARCH REPORT

PCT/US03/41600

**Continuation of B. FIELDS SEARCHED Item 3:**  
CAPLUS, MEDLINE, WEST, BIOSIS  
Search terms: inventor name, scfv, hinge, cysteine, fusion protein, CD19, CD3, deleted hinge, altered hinge, IgG1, IgA, IgE, disulfide stabilized, constnat region.